ORALS

001

ADVANCES IN UNDERSTANDING THE MOLECULAR BASIS OF BONE REMODELLING: PAST, PRESENT AND FUTURE

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The first concepts of intercellular communication in bone came with the idea that osteoblasts control the formation and activity of osteoclasts. The discovery of osteoprotegerin and the RANKL/RANK signaling mechanisms established this pathway as essential for normal bone resorption. It is clear now that there are many more local communication systems operating in bone to maintain bone balance. In bone remodeling, osteoclasts resorb a certain amount of bone and die, leaving the space to be filled by mesenchymal cells that differentiate into osteoblasts and form bone. Growth factors released from resorbed bone matrix can contribute to preosteoblast differentiation and bone formation. It is recognised now also that PTHrP generated locally in bone is a crucial physiological regulator of bone remodeling, perhaps providing a mechanism by which preosteoblasts themselves, growing in the resorption space, can communicate through cell contact and paracrine signalling mechanisms. One such signalling mechanism is production of ephrinB2, regulated by PTHrP in osteoblasts, acting on receptor EphB4 in the same population to promote bone formation, and regulating the filling of BMUs by new bone. Osteocytes can sense the need for bone repair by detecting damage and pressure changes, and signalling to surface cells to respond appropriately, either to enhance resorption, or to influence formation by changing the release of the osteocyte-derived formation inhibitor, sclerostin. Sclerostin is emerging as a powerful inhibitory influence on bone formation in remodelling, acing as an inbuilt braking system. There is increasing evidence also that osteoclasts in the BMU generate activity that contributes to bone formation. It is possible also that osteoclasts, transiently activated by PTH can contribute to the coupling of bone formation to resorption by producing activity that influences preosteoblast participation in bone formation.

While all this information has been gathered over the last few years, major new influences on bone remodelling have emerged to be fitted into the picture, particularly those from the central nervous system, and serotonin derived from the small intestine as an inhibitor of bone formation.

002

GENETIC DETERMINANTS OF LOCOMOTER DISEASE

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M any -if not all- common diseases including locomoter diseases such as osteoporosis and osteoarthritis, have strong genetic influences and therefore intense efforts are ongoing to identify the underlying genetic variants. Knowledge of these variants can help in understanding the disease process and might benefit development of interventions and diagnostics. Previously, genome wide linkage searches and candidate gene studies have been pursued to this end, but of these only candidate gene studies have been successful to some extent, but not without controversy. Genome Wide Association (GWA) studies have now become the standard approach to uncover the strongest genetic effects of common variants on a genome-wide scale. The GWA approach builds upon the availability of extensive data on human genetic variation, substantial improvements in genotyping technology including very high-density SNP arrays, and accessibility of biobanks of large population cohorts with DNA and phenotype information.

In the past few years GWA has proven to be widely successful for several complex diseases in discovering novel and common risk genes mainly in Caucasian populations. For osteoporosis and osteoarthritis several large GWA studies are underway, and the first results have been published. Interestingly, experience so far has shown that GWA -in general- identifies DNA variants rather then genes, and that the effects seen for the common variants are modest, e.g., with Odds Ratios ranging from 1.1 –1.7 and explained variance of combined variants being a few %. On the one hand these features shed interesting light on how human genes are regulated and what common genetic variation is tolerated throughout evolution, but on the other hand also pose serious challenges in translating the results of GWA to clinical practice at this stage. Much effort is therefore focussed on linking GWA findings to actual genes and to establish the biological mechanism of associations. In addition, efforts will be shifting from analysing common variants, mostly single nucleotide polymorphisms (SNPs), to the more rare DNA variants using deep sequencing, and also include other types of DNA sequence variation.

An important part of the process of GWA analysis is the testing of identified variants in international consortia with very large collections of DNA samples with a certain phenotype. The GENOMOS consortium has played such a role in

the field of osteoporosis and has in the "pre-GWA" era already identified (and refuted) associations of well known candidate genes. Similarly, the newly formed TREAT OA consortium will play such a role in the field of osteoarthritis. Cross talk between such efforts will also be necessary to identify pleiotropic effects of gene variants, several examples of which have already been seen. Together with genetic studies on more rare syndromes, the GWA approach is likely to help in clarifying the genetic architecture of complex traits and diseases including locomoter diseases.

003

GENETIC MODELS OF BONE DISEASE USING ENU MUTAGENESIS

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With the completion of the mouse genome sequence, a key goal for functional genomics is the creation of a series of mutant alleles for every mammalian gene. Mouse genetics has developed a comprehensive basket of tools for generating mutations, including the use of the chemical mutagen, *N*-ethyl-*N*-nitrosourea (ENU). ENU introduces point mutations into the mouse genome providing a wide variety of potential functional changes in a gene that might mirror human genetic variation from loss of function, partial loss of function to gain of function mutations and dominant-negatives. Mice are mutagenised with ENU and their progeny carrying ENU mutations are screened for disease phenotypes of interest. In this phenotype-driven approach no *a priori* assumptions are made about the underlying genes involved and thus ENU is potentially a very powerful tool for revealing novel disease pathways. An even greater challenge for mouse genetics will be the determination of phenotypic outcomes for each mutation and the identification of disease models. A vital element of this endeavour will be to develop standardised phenotyping platforms that allow for reproducibility of test outcome over both time and place. The EUMORPHIA programme, funded by the European Commission, and comprising a consortium of 18 research institutes around Europe, has developed a new robust primary screening strategy, EMPReSS (European Mouse Phenotyping Resource for Standardised Screens). This primary screen incorporates over 150 SOPs, many validated on a cohort of inbred strains across a number of laboratories. EMPReSS covers all of the major body systems, including a variety of phenotyping platforms that bear upon bone disorders. We have been employing ENU mutagenesis harnessed to developments in mouse bone phenotyping, including the standardised EMPReSS phenotyping platforms, to identify new mouse mutant models of bone disorders. We have undertaken screens for both dominant and recessive disorders, and have identified a number of novel mutants that will be elaborated and discussed.

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(2) HOUGH, T.A. et al. (2007) A novel mouse model of autosomal semi-dominant adult hypophosphatasia has a splice site mutation in the tissue non-specific alkaline phosphatase gene Akp2. J. Bone Mineral Re

(3) BARBARIC, I. et al. (2008) An ENU-induced mutation in the Ankrd11 gene results in an osteopenia-like phenotype in the mouse mutant Yoda. Physiol. Genomics 32: 311-321

004

CONTRIBUTIONS OF THE FTO GENE TO FRACTURE RISK

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Common variants in the Fat mass and Obesity-associated (FTO) gene are associated with body weight, and body weight is associated with bone mineral density (BMD). This study sought to test the hypothesis that common polymorphic variations at the FTO gene are associated with BMD and thus with fracture risk.

Haplotype tagging single nucleotide polymorphims (in order rs1421085, rs1558902, rs1121980, rs17817449, rs9939609 and rs9930506) of the FTO gene was determined in a sample of 1294 women aged 60+ years as at 1989. Anthropometric data were obtained from all women. BMD at the spine and femoral neck, lean mass and fat mass were measured by DXA (GE-Lunar Corp, WI, USA) at baseline. Symptomatic fracture incidence was ascertained during the

follow-up period of 1989-2007. Haplotypes of the FTO gene were constructed by the EM algorithm and analyzed for association with BMD and fracture risk by the general linear model in the SNPassoc program within the R system.

The distribution of the 6 SNPs was consistent with Hardy-Weinberg equilibrium. In univariate analysis, the risk of hip fracture was increased among women carrying the minor alleles of the SNP rs1121980 (RR 1.39; 95% CI: 1.04-1.87) and rs9930506 (RR 1.36; 95%CI: 1.01-1.84). In comparison to those with the haplotype TTGTTA (relative frequency 51%), women carrying the haplotype CAAGAG (37.2% in the sample) had lower BMI (-0.42 kg/m^2; p=0.04), lower lean mass (-0.39 kg; p<0.0001) and lower fat mass (-0.4 kg, p<0.0001) after correcting for age. The haplotype was also associated with an increased risk of hip fracture (RR 1.42; 95%CI: 1.01-2.04) after adjusting for age and femoral neck BMD. Moreover, the haplotype TTGTAA (relative frequency 3%) was also associated with lower BMI, lean mass, fat mass and BMD but was not a fracture risk.

In the present study, a haplotype-based analysis was more powerful than a SNP-based analysis in the detection of association. These results support the hypothesis that common genetic variation at the FTO gene is modestly associated with fracture risk in women independent of its association with BMD and obesity-related phenotypes.

005

A VARIANT OF HUMAN AROMATASE PREDICTS INCREASED CIRCULATING ESTRADIOL AND BONE HEALTH IN POST-MENOPAUSAL WOMEN

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The CYP19A1 gene encodes the enzyme aromatase, which is responsible for the final step in the biosynthesis of estrogens. Work from our group has shown that a microsatellite in intron 4 is associated with bone density and estrogen levels. Moreover a threonine to methionine missense mutation at amino acid position 201 in exon 6 of the aromatase gene causes a 5 fold increase in aromatase activity in an in-vitro assay. In this study we have characterized the effect of this variant on a large population of elderly women. A cohort of 1,257 post-menopausal Western Australian women aged 75 ± 2.5 yr in 1998 were recruited from the Western Australian electoral roll. Bone mineral density (BMD) at the hip and spine sites was measured by DXA (Hologic QDR 4500). Serum estradiol was measured by RIA (Orion Diagnostica, Finland). Genotyping of the $T^{201}M$ site was by Taqman (Applied Biosystems, Foster City, CA). Spinal deformity was calculated from morphometric x-ray calculations. The frequencies of the genotypes in the population were CC = 0.83, CT = 0.16, TT = 0.01. Using a dominant model the variant (T) was associated with increased estradiol for the heterozygote and rarer homozygote combined (CC: 25.5 ± 14.9 pmol/L vs. CT, TT: 38.3 ± 27.4 pmol/L; P = 4.5 $x10^{-21}$). In addition, the variant allele was associated with increased BMD at the total hip (CC: 806 ± 126 mg/cm^2 vs. CT, TT: 831 ± 129 mg/cm^2; P = 0.015), femoral neck (CC: 686 ± 104 mg/cm^2 vs. CT, TT: 706 ± 109 mg/cm^2; P = 0.021). This variant was also associated with a reduction in spinal deformity (L4 – T12) (CC: 37.6% vs. CT, TT: 21.1%; P = 0.012). We found that carriers of the T allele (17% of the population) show increased circulating estrogen, increased DXA BMD and reduced spine deformity. The observed associations are in keeping with the molecular effects of the nucleotide change previously characterised *in vitro*. These findings serve as the basis for further examination of the regulatory mechanisms of aromatase expression in human bone tissue and suggests that this variant may be of clinical use in predicting propensity to disease.

006

SEXUAL DIMORPHISM IN THE ASSEMBLY OF METAPHYSEAL CORTICAL AND TRABECULAR ARCHITECTURE

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Little sex-difference exists in bone size or mass before puberty. At the completion of growth, males have larger bone size than females but sex differences in cortical and trabecular architecture are poorly defined. We studied 128 Caucasian subjects (male/female = 68/60) aged from 5 – 18 yrs using high-resolution pQCT at the distal metaphyses of the radius and tibia (Xtreme CT, Scanco, Switzerland). There was no difference in bone size, total volumetric bone mineral density (vBMD), cortical thickness or trabecular morphology between sexes before puberty. While bone size increased across the pre- and pubertal years of life, trabecular bone volume fraction (BV/TV) was independent of age

at the distal radius, and increased modestly at the distal tibia in both sexes due to increased trabecular thickness (Fig). Cortical thickness and cortical vBMD increased only in late puberty (Tanner stage III - IV) at both sites. At late and post-puberty (Tanner stage III - V) sex-differences were present in favor of the male in bone size, cortical area and trabecular BV/TV (0.161 ± 0.018 vs. 0.145 ± 0.023, $p < 0.01$) but in favor of the female in cortical vBMD (830 ± 61 vs.744 ± 64 mg HA/cm^3, $p < 0.01$) at the distal tibia, probably because of earlier consolidation and reduced cortical porosity in females (Fig). Similar pattern of sex-differences was observed at the distal radius (data not shown). In summary, radial and tibial metaphyseal growth is mainly in macroarchitecture (bone size and cortical area). Trabecular architecture are largely independent of age while cortical thickness increases late in puberty. We infer that at the completion of growth, the sex difference in bone strength mainly is due to differences in bone macrostructure (size, and cortical area); and trabecular morphology differs little by sex.

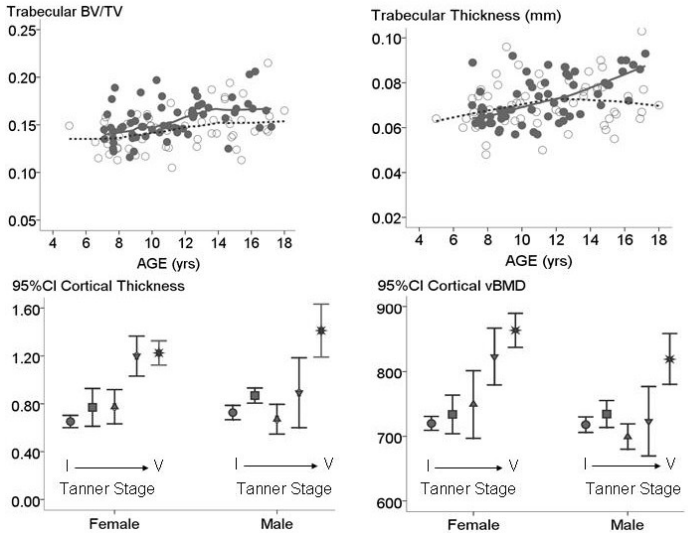

Fig. Trabecular and cortical traits at the distal tibia. Solid lines and dots for males and open for females in the upper panel. Growth in cortical thickness and vBMD through puberty is shown in the lower panel.

007

HOW LONG FOLLOW-UP IS REQUIRED TO ACHIEVE AN ACCURATE ESTIMATE OF 10-YEAR ABSOLUTE RISK OF FRACTURE AMONG THE ELDERLY?

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Estimation of 10-year absolute fracture risk for older populations is now recommended by the World Health Organization (WHO). These estimates should ideally come from country-specific prospective studies that follow representative members of the community for a long time. Given practical and resource constraints, cohort studies usually follow participants for a shorter interval (typically 4-7 years) and extrapolate their results to generate 10-year predictions. The most widely used statistical methods are based on exponential distribution of fracture risk and using Poisson regression (as used in FRAX study by the WHO Collaborating Group). However, the length of follow-up necessary to achieve an accurate estimate of 10-year risk is not clear.

The original cohort in the European Prospective Investigation into Cancer-Norfolk study comprised 25,312 men and women aged 39-79 years living in the general community in Norfolk, United Kingdom, recruited in 1993-1997 and followed-up for incident fractures to 2007 (mean follow-up 11.4 ± 1.3 years). Sex-specific Poisson regression models adjusting for age, history of fracture, height, body mass index, smoking and alcohol consumption were employed to obtain 10-year absolute fracture risks in 10 different sub-cohorts with one year added interval of follow-up (the original cohort was re-arranged to produce 10 cohorts with follow-up period of 1, 2, 3, …, and 10 years; incident fractures after the follow-up period were censored for each cohort).

While 758 fractures were observed in the first 10 years of follow-up among EPIC-Norfolk participants, models with 5, 6, 7, 8, 9, and 10 years of follow-up, respectively, predicted this number to be 423, 491, 569, 638, 685, and 761 fractures. Choosing arbitrary cutoffs for definition of high-risk (10-year risk of >6% in <65-years-old and >12% in ≥65-years-old participants to reflect the fracture incidence in the original cohort) and using the model with 10-years

follow-up as the gold standard, the sensitivity of the model with 6 years follow-up was only 49.2%. This number increased for models with 7, 8, and 9 years of follow-up, respectively, to 55.8%, 79.6%, and 91.8%.

This study suggests that follow-up periods of at least 8 years are needed to obtain sufficiently accurate estimates of 10-year fracture risk.

008

RACIAL DIFFERENCES IN MACRO AND MICROARCHITECTURE OF THE APPENDICULAR SKELETON IN YOUNG CHINESE AND CAUCASIAN FEMALES.

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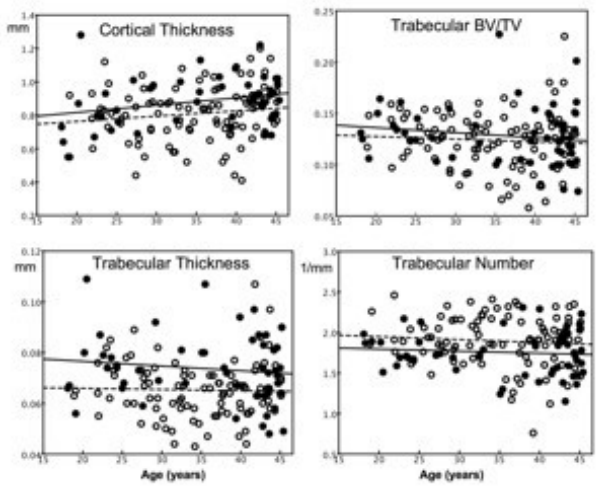
Hip and forearm fracture rates are lower in Asians than Caucasians. To identify the racial differences in macro- and microstructure that may partly account for racial differences in bone fragility we studied 61 healthy premenopausal Chinese and 111 Caucasian women aged 18 to 45 years (mean 35.8 vs. 34.8 years old) living in Melbourne using high-resolution peripheral quantitative computed tomography (HR-pQCT, Scanco Medical AG, Bassersdorf, Switzerland) at distal radius and distal tibia. Chinese were shorter, and leaner. Distal radius total cross-sectional area (CSA) was 14.3% less due to a 18.0% smaller trabecular bone area (6.2% and 9.0% after height and weight adjustment, $p < 0.05$). Cortical area was no different but cortical thickness was 8.8% greater in Chinese. Cortical vBMD was 2.8% higher in Chinese ($p<0.01$). Total vBMD was 10.3% higher in Chinese because of the higher cortical thickness and density ($p<0.01$). Trabecular vBMD and bone volume/tissue volume (BV/TV) did not differ by race but trabeculae were fewer (-7.0%, $p<0.01$) and thicker (10.8%, $p<0.01$) in Chinese (Fig). Similar results were found at the distal tibia. Lower fracture risk in Chinese may be partly due to Chinese women have thicker cortices and trabeculae in a smaller bone – more bone within the bone than Caucasians.

Fig. Cortical thickness, trabecular BV/TV, number and thickness at distal radius in Chinese (filled dots, solid line) and Caucasian (open dots, dashed line) women as a function of age.



009

GLUCOCORTICOID-INDUCED CORTICAL BONE LOSS IS MEDIATED BY OSTEOBLAST-CONTROLLED ACTIVATION OF BONE RESORPTION

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INTRODUCTION: The mechanisms of glucocorticoid (GC)-induced bone loss are poorly understood. We investigated the role of the osteoblast in mediating GC effects on bone using a transgenic mouse model in which GC signalling is abrogated in mature osteoblasts on the pre-receptor level by transgenic (tg) overexpression of 11beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2).

METHODS: Eight week-old male tg and wild-type (WT) mice (n=9-10/group) received weekly s.c. implants of slow-release pellets containing either 1.5 mg corticosterone (CS) or placebo for a total of 4 weeks, after which tibiae and lumbar vertebrae were analysed by micro-CT and histomorphometric analysis. Serum levels of tartrate-resistant acid phosphatase 5b (TRAcP-5b) and pro-collagen type I N-terminal peptide (PINP) were determined by EIA on days 0, 7, 14, 21 and 28.

RESULTS: *Micro-CT analysis:* Compared to controls, CS induced a significant reduction in cortical thickness (vertebrae: -13%, $p<0.005$; tibia: -9%, $p=0.12$) and endosteal perimeter (tibia: -14%) in WT mice but not in tg animals. Periosteal perimeter was similar in CS-treated WT animals and controls but reduced by 5% ($p=0.048$) in CS-treated tg mice compared to untreated tg controls. Trabecular BV/TV, trabecular number and trabecular separation were unaffected in CS-treated tg and WT animals. *Histomorphometry:* CS treatment increased osteoclast surface (+57%; $p=0.003$) and osteoclast number/bone surface (+36%, $P= 0.02$) in WT mice with no change in tg mice. Osteoblast surface remained unchanged with a trend towards lower values in both CS-treated WT and tg animals. *Biochemistry:* Between day 0 and day 21, serum TRAcP-5b levels decreased in untreated WT (-17%), untreated tg (-24%) and CS-treated tg mice (-14%). In contrast, serum TRAcP-5b levels increased in CS-treated WT mice (+17%). Serum PINP levels decreased in untreated WT (-14%) and tg (-27%) animals and were suppressed in CS-treated WT (-74%) and tg (-69%) mice. Multivariate analysis revealed a significant interaction between CS-treatment and genotype for TRAcP-5b but not for PINP.

CONCLUSION: GC-induced cortical bone loss appears to be mediated through osteoblast-controlled activation of bone resorption.

010

ROLE OF BONE DENSITY AND CLINICAL RISK FACTORS IN MORTALITY RISK FOLLOWING ALL OSTEOPOROTIC FRACTURES IN ELDERLY WOMEN AND MEN: AN 18-YEAR PROSPECTIVE STUDY FROM DUBBO OSTEOPOROSIS EPIDEMIOLOGY STUDY

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Little is known about the risk factors for post-fracture mortality. Low bone mineral density (BMD) has been associated with both non-trauma mortality and fracture risk, but its role in post-fracture mortality is unclear. The aims of this study were to examine 1) predictors for mortality following all fragility fractures and 2) the effect of fracture over and above low BMD on mortality.

Subjects aged 60+ participating in the Dubbo Osteoporosis Epidemiology Study with incident fractures (April 1989 - May 2007) and with BMD, postural stability, quadriceps strength, co-morbidities and lifestyle data collected around the fracture time were included. The effect of fracture on mortality over-and-above low BMD was assessed in 347 women and 129 men who were matched 1:1 by age and BMD to non-fracture participants.

There were 452 women and 162 men with incident fractures followed by 202 deaths in women and 97 in men. Increasing age, quadriceps weakness and re-fracture independently predicted mortality risk in both sexes. Low BMD, having smoked and increased sway were independent mortality predictors in women, and physical inactivity and fewer falls in men. Despite more cardiovascular illnesses in women who died and more neurological and respiratory diseases in men who died, comorbidities were not associated with mortality.

Population attributable risk for premature mortality was greatest for low BMD in women (18%). Re-fracture contributed 13 % and 14% in women and men respectively. Ever having smoked contributed 10% in women while being in the worst quartile of any of the other factors accounted for ≤ 7%.

Women with low BMD had similarly increased mortality to the matched women irrespective of fracture [standardised mortality ratios: 1.44 (1.21-1.71) and 1.47 (1.23-1.75)]. However male fracture participants had higher mortality risk than their age-matched non-fracture counterparts [OR: 1.95 (1.15-3.30)].

This study suggests that fracture is a signal for an underlying increased mortality risk, particularly in women where low BMD appear to play an important role. In men, factors surrounding the fracture event may be more relevant to the associated premature mortality. The causes and prevention of this premature mortality require more urgent study.

011

IN VIVO QUANTIFICATION OF AGE-RELATED CHANGES IN HUMAN CORTICAL BONE THICKNESS AND POROSITY BY HR-PQCT

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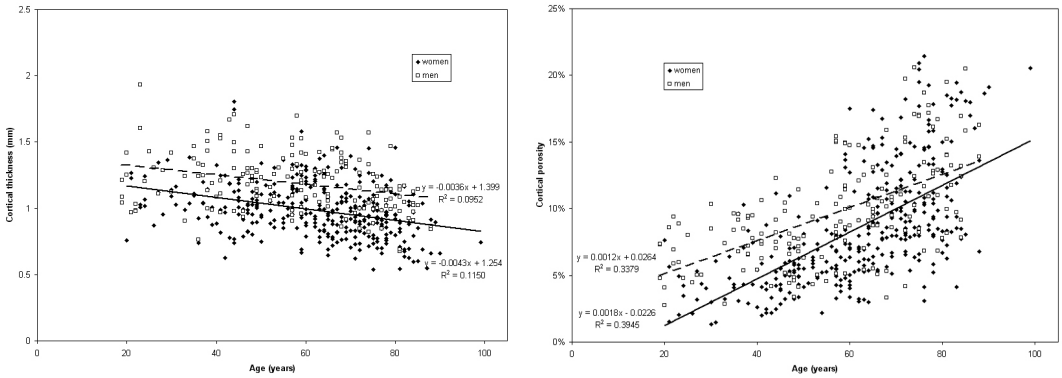
Aging has been shown to cause many changes in the structural properties of bone such as reduced cortical thickness (Ct.Th) and increased cortical porosity (Ct.Po) which may increase the risk of fracture. The purpose of this study was first to establish the ability of high resolution peripheral quantitative computed tomography (HR-pQCT) to quantify Ct.Th and Ct.Po *in vivo* through fully automated analysis, and second, to determine how Ct.Th and Ct.Po change with age in men and women. Automated image registration and cortical segmentation (1) procedures were used to obtain direct measurements of Ct.Th and Ct.Po, making it practical for use on large populations. As a preliminary validation of the procedures, 8 human cadaver forearms (5 women, 3 men, ages 68-93 years, mean 84) were scanned at the standard measurement site using HR-pQCT (XtremeCT, Scanco Medical, Switzerland) followed by scanning of the dissected radii by the gold standard, µCT (vivaCT 40, Scanco Medical, Switzerland). The nominal isotropic resolution for the HR-pQCT and µCT were 82µm and 19µm, respectively. A linear regression analysis was performed relating the HR-pQCT measurements to the µCT measurements giving R^2 values of 0.97 for Ct.Th and 0.60 for Ct.Po. The automated analysis was then applied to distal radius HR-pQCT scans from a sample (N=510, 339 women, 171 men, ages 20-99 years) of the Calgary cohort of the Canadian Multicentre Osteoporosis Study (CaMos) , a population-based study of over 9000 women and men in 9 urban centres across Canada. Linear regression analyses were performed and preliminary findings indicate Ct.Th was moderately associated with age in both women (R^2=0.12) and men (R^2=0.10) whereas Ct.Po showed a stronger correlation with age (women: R^2=0.39 and men: R^2=0.34) (Figure). In summary, this automated method provides a tool to measure Ct.Th and Ct.Po *in vivo* using HR-pQCT, and has been applied to a large cohort to identify changes in Ct.Th and Ct.Po with age in men and women. Future work will identify the functional significance of these changes by applying the finite element method.



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012

BIOLOGY OF FRACTURE HEALING AND EFFECT OF OSTEOPOROSIS THERAPIES

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With the advent of effective pharmaceutical treatments for osteoporosis and metabolic bone disease, there is an active effort to investigate the effects of similar compounds to bone repair. As well as questions relating to the safety of osteoporosis treatments in patients sustaining osteoporotic fracture, optimisation and combinations of these therapies are being explored as an adjunct to fracture healing.

There is a further layer of complexity in bone repair as compared to the already complex events that regulate bone remodelling. In addition to the processes that modulate bone formation and resorption, bone repair involves the recruitment of mesenchymal cells and the vasculature in a coordinated way to re-establish the bone microenvironment.

Riggs and Parfitt have suggested classifying osteoporosis drugs according to their principle action, ie anabolic or anti-catabolic. It is similarly useful to expand on this concept and think of bone repair as a system of coordinated anabolic and catabolic responses. The anabolic response can be further divided into non-specific anabolism (wound repair) and specific (bone) anabolism. One must also remember that agents can affect both anabolic and catabolic processes, and the relative effects are situation and time dependent.

Thus pharmaceutical agents can be classified according to their effects on the various parts of the process. For example, PTH is a very specific anabolic, ie it acts on committed and mature bone cells. PTH has no known effect on non-specific anabolism (ie wound repair phenomena of cell recruitment and angiogenesis). PTH also indirectly increases catabolism. Bisphosphonates are anti-catabolic but eventually have indirect effects which reduce bone formation via remodelling. At high doses at least experimentally bisphosphonates can modulate effects on non-specific anabolism such as angiogenesis. This classification system can be applied to novel therapies which are emerging such as RANKL or SOST inhibition such that the likely effective scenarios for their application in bone repair can be deduced and then tested.

Finally, there are early clinical data emerging on the effects of osteoporosis agents on bone repair, but these data are currently quite limited.

013

BONE MORPHOGENETIC PROTEINS (BMPS) AND FRACTURE REPAIR

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While the remarkable regenerative capacity of bone has been known for centuries, the idea that the signals responsible for bone healing were produced by bone cells and stored in bone matrix was first hypothesized by Marshall Urist in the1960s and given the name bone morphogenetic protein (BMP). We now know that BMP activity resident in bone matrix is the product of at least 3 separate genes, BMPs 2, 4 and 7, and that these genes belong to a very large and functionally diverse gene family that also includes TGF- b s, activins, and myostatin, among others. As mice lacking BMP2 or BMP4 die early in embryogenesis prior to formation of the skeleton, and mice lacking BMP7 die immediately after birth, we chose to perform conditional inactivation of each of these BMP genes in limb mesenchyme at the onset of skeletogenesis as a way to evaluate the requirement for specific BMPs in fracture repair. Using this approach, we found that mice lacking limb expression of either BMP4 or BMP7 produce normal skeletons and are able to successfully mount a fracture healing response. In contrast, mice lacking limb expression of BMP2 appear to have normal limb skeletons at birth but are unable to maintain bone formation after birth as evidenced by 100% incidence of spontaneous fractures of limb bones as the mice age. Molecular analysis of the fracture healing response in bones lacking BMP2 showed that periosteal cells at the fracture site failed to proliferate in response to fracture, and the mesenchymal progenitors present were unable to differentiate into chondrocytes and osteoblasts, leading to a failed healing response. From these studies we conclude that BMP2 is required for the initiation of fracture repair and that BMP4 and BMP7 are unable to substitute for the loss of BMP2 during fracture healing.

014

STEM CELLS AND FRACTURE REPAIR

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The fracture healing process is impaired in 10-20% of the fractures causing disability, economic cost for the individual and the society and even death. Current therapies for non-unions consist mostly on bone grafts that are associated with complications, inconsistent outcomes and sometime unavailability of sufficient tissue. Adult bone marrow mesenchyme stem cells (MSC) are capable to differentiate into several cells including osteoblasts and chondrocytes. Research in MSC has become one of the investigative objectives for regenerative medicine. The use of MSC to treat non-unions is attractive as: 1) it would implement a reparative process that should be in place but it occurs to be defective; 2) MSC regenerative abilities would be needed only for the repairing time that is relatively brief; 3) MSC beneficial effects would be assessable using radiological analyses. MSC represent an appealing approach for treating non-unions and some beneficial effects were reported by uncontrolled clinical studies. It is plausible that due to their intrinsic multipotentiality, MSC have several and distinct reparative actions. Before clinical trials begin, critical animal studies are needed to determine how MSC are recruited to the fracture site, their survival for the time of the healing, their repair effectiveness, the mechanisms through which exert their actions and the beneficial effects of combining MSC with osteochondro-inductive growth factors. My laboratory has developed several studies to provide a comprehensive approach to address these questions. We have employed cutting-edge technologies to achieve our purposes including genetically modified mice, bioluminescence, micro-CT, biomechanical testing, computational analyses for modeling biological processes. In immunologically intact mice with a stabilized tibia fracture, we determined: 1) the dynamic migration of transplanted MSC to the fracture site; 2) the prerequisite of the chemokine receptor, CXCR4, for MSC migration; 3) the endosteal niche within the callus for the MSC engraftment; 4) the ability of MSC to express BMP-2, an essential initiator of the fracture healing 5) the improvements in callus material properties induced by MSC; 6) the systemic and local modulatory effects of MSC on the injury-induced inflammatory responses; 7) the contributions of MSC engineered with insulin-growth factor-I to the healing process. Understanding the distinct regenerative contributions of MSC to the fracture repair process will allow to design novel MSC-based therapies for patients with non-unions.

015

NUCLEOTIDES AND REGULATION OF BONE CELL FUNCTION

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It is now well recognised that extracellular nucleotides (chiefly ATP, ADP, UTP & UDP), signalling via the P2 receptors, are involved in a wide number of biological processes in both neuronal and non-neuronal tissues. P2 receptors are subdivided into the P2X (ion channel) and P2Y (GPCR) families. In recent years, the role of extracellular nucleotides in the regulation of bone cell function has received considerable attention. Work in several laboratories, including our own, has shown that numerous P2 receptor types are expressed by bone cells (osteoblasts: $P2X_{1\text{-}7}$ and $P2Y_{1,\ 2,\ 4,\ 6\ \&\ 12\text{-}14}$ receptors; osteoclasts: $P2X_{1\text{-}4\ \&\ 7}$ and $P2Y_{1,\ 2,\ 4,\ 6\ \&\ 12\text{-}14}$ receptors). In rat osteoblasts, the pattern of P2 receptor expression changes markedly with cell differentiation. For example, $P2X_2$ and $P2X_5$ are highly expressed in immature, proliferating cells, whereas $P2X_{3,\ 4,\ 6\ \&\ 7}$ and $P2Y_{2,\ 6\ \&\ 14}$ are upregulated in mature cells. $P2Y_2$ is particularly strongly expressed in bone-forming osteoblasts. Moreover, UTP (1-100mM), an agonist at $P2Y_2$, is a potent, selective inhibitor of bone matrix mineralisation and osteoblast alkaline phosphatase expression; ATP, the 'universal' agonist, acts similarly. Consistent with this finding, $P2Y_2$ -deficient mice exhibit significant increases in bone mineral content (and also trabecular bone volume). It is likely, however, that there is also a non-receptor mediated component to the inhibitory action of extracellular ATP/UTP on mineralisation, due to pyrophosphate generated by the action of ecto-nucleotide pyrophosphatase / phosphodiesterases (which are expressed by osteoblasts) on nucleotide triphosphates. ATP (0.2-20mM) additionally exerts a significant stimulatory action on the formation and pit-forming activity of osteoclasts; this effect is also observed with ADP and 2-methylthioADP, suggesting involvement of the $P2Y_1$ or $P2Y_{12}$ receptors. Lastly, we and others have good evidence for constitutive, vesicular release of ATP from healthy osteoblasts in vitro, in biologically significant amounts. These findings support the notion that extracellular ATP could have a dual action as a paracrine/autocrine signalling agent in bone, stimulating resorption, whilst inhibiting mineralisation.

016

CYTOKINES THAT SIGNAL THROUGH GP130 AND THEIR ROLES IN BONE BIOLOGY

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The receptor subunit gp130 is utilized by a wide range of cytokines, including IL-6, IL-11, LIF, cardiotrophin 1 (CT-1) and oncostatin M (OSM), which form unique signalling complexes that also involve ligand-specific receptors. Many of these cytokines have been shown to have strikingly similar effects in bone. Specifically they all act on receptors in osteoblasts in co-culture systems to increasing osteoclast formation. More recently, significant roles for these cytokines in osteoblast differentiation and bone formation during growth and remodelling have been described.

Some of this evidence is derived from studies of knockout mice. Deletion of gp130 is neonatal lethal, with significantly increased osteoclast formation as well as reduced osteoblast numbers in embryonic bone. In contrast, analysis of adult mice with a specific mutation in the gp130 subunit revealed increased osteoblast formation, which was coupled through an IL-6 dependent pathway to a high level of osteoclast formation.

In contrast, adult mice that lack IL-11R, OSMR, CT-1 or LIF all exhibit a low level of osteoblast formation and low rate of bone formation. Furthermore, in the case of OSMR and CT-1 deletion, this impaired osteoblast differentiation of osteoblasts is cell lineage autonomous. Further analysis of these cytokines in cell culture systems have revealed specific stimulatory actions on osteoblast differentiation, mineralisation and inhibitory influences on adipogenesis. *In vivo* administration over calvariae of wild type mice of either OSM or CT-1 also increased bone formation. Furthermore, immunohistochemical studies have demonstrated CT-1 protein in osteoclasts, while its receptor is expressed in osteoblasts. In contrast OSM and its receptor have been identified within osteoblasts, bone lining cells and osteocytes, confirming specific roles for these two cytokines in the local control of both bone resorption and bone formation. Taken together these results indicate multiple roles for gp130 cytokines in controling osteoblast and osteoclast function, including paracrine roles that may mediate signaling between these two cell types during bone growth and remodelling.

017

SEX STEROID HORMONES EXHIBIT OSTEOPROTECITVE EFFECTS THROUGH NUCLEAR RECEPTORS EXPRESSING IN BONE CELLS

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Sex steroids, estrogens and androgens, display an osteoprotective effect and prevent bone loss associated with post-menopausal osteoporosis. However, the molecular mechanism of how this is accomplished remains to be elucidated. We have generated Cathepsin K-Cre recombinase knock-in mice (Ctsk-Cre) to generate osteoclast specific conditional knock-out mice. In this mouse, cre recombinase was specifically expressed in bone tissue, especially in osteoclasts. We selectively ablated androgen receptor (AR) and estrogen receptor (ERα) in differentiated osteoclasts using Ctsk-Cre mice mating with AR floxed mice and ERα floxed mice ($AR^{\Delta Oc/Y}$ and $ER\alpha^{\Delta Oc/\Delta Oc}$). $ER\alpha^{\Delta Oc/\Delta Oc}$ females and $AR^{\Delta Oc/Y}$ males exhibited clear bone loss in plain X-ray and 3D-CT, similar to the osteoporotic bone phenotype. Also in DEXA, femurs of $ER\alpha^{\Delta Oc/\Delta Oc}$ females and $AR^{\Delta Oc/Y}$ males showed low bone mineral density. Bone histomorphometric analysis revealed a significant increase in osteoclast surface, osteoclast number and eroded surface in $AR^{\Delta Oc/Y}$ males with increased MAR and BFR. These results showed that $ER\alpha^{\Delta Oc/\Delta Oc}$ females and $AR^{\Delta Oc/Y}$ males exhibit high turnover osteoporotic phenotypes (Kawano et al., PNAS 2003; Nakamura et al., Cell, 2007). Currently, we are characterizing the role of AR and ERs in osteoblasts by means of Col Ia-Cre tg mice.

(1) Kawano, H. et al. Suppressive function of androgen receptor in bone resorption. Proc Natl Acad Sci USA. 100(16), 9416-21 (2003)

(2) Nakamura, T et al. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. Cell 130(5), 811-23 (2007)

018

ESTROGEN RECEPTOR A IS A CRITICAL CONTRIBUTOR AT MULTIPLE SITES TO BONES' ADAPTIVE RESPONSE TO MECHANICAL LOADING

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Bones' ability to withstand the loads of everyday activity without fracture is achieved through resident bone cells using loading-induced strains in the bone tissue as a stimulus to adjust bone mass and architecture to achieve and maintain a "target strain environment". Only short exposure to dynamic strain is necessary to stimulate the cascade of events that prevent bone resorption and/or stimulate bone formation. Early events following exposure to dynamic strain in resident bone cells include calcium fluxes; stimulation of the ERK pathway; production of prostaglandins and nitric oxide; activation of the canonical Wnt pathway; altered expression of a large number of non-bone specific genes; depression of sclerostin production and increased secretion of Insulin-like Growth Factors. Estrogen Receptor α , independently of estrogen, is involved up and down stream of ERK activation; in the production of nitric oxide but not prostaglandins; translocation of β-catenin to the nucleus; the regulation of a large number of genes and activation of the IGFR. The conservational and potentially osteogenic response to bone loading is substantially reduced in animals lacking ERα,but not ERβ.

The most widespread failure of bone's mechanically-adaptive response to maintain the skeleton sufficiently robust to withstand everyday loading without fracture occurs in post-menopausal osteoporosis in women and age-related osteoporosis in men. In both sexes the level of bone loss is associated with reduced bioavailable estrogen. Estrogen, but not strain, appears to regulate the ER number in osteoblasts and osteocytes, which in the estrogen replete state is only some 200-300/cell.

We hypothesise that the etiology of osteoporosis is substantially due to declining estrogen levels leading to insufficient ERα in resident bone cells to adequately respond to strain. This produces a similar downstream situation as the absence of strain, as in disuse. The result is also similar – bone loss towards a genetically determined minimum. Since this bone loss is accompanied by continued loading the consequence is increased incidence of fragility fracture.

019

WINNING THE BATTLE AGAINST CHILDHOOD PHYSICAL INACTIVITY: THE KEY TO BONE STRENGTH?

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The British Columbia (Canada) Provincial Health Officer recently published a report that stated "

Habits of living, nutrition and exercise patterns become established during the growing years. It is clear from the global experience that opportunities exist within the school setting to significantly and positively influence many domains of youth health."

Childhood and adolescence are also key times for the development of a healthy skeleton and the contributions of physical activity to bone health are well known. Given that children spend 30 of their waking hours in school each week, schools may provide the ideal vehicle to deliver physical activity models that target overall health – including bone health. In this presentation I will introduce effective school-based bone health intervention models and strategies for dissemination.

Our understanding of the complex nature of bone's adaptation to physical activity has evolved over the years. Traditional methods of assessing bone mass at any age - such as dual energy x-ray absorptiometry (DXA) – fail to represent the complex, hierarchical 3- dimensional structure of bone. Novel imaging techniques such as peripheral quantitative computed tomography and extreme CT technology can safely and precisely achieve this and assess bone structure and strength. Thus, I will also introduce innovative techniques for evaluating the 3-D structure of bone and bone's adaptation to physical activity interventions.

020

PROTEOLYTICALLY PROCESSED REPRESSOR GLI3 INHIBITS BONE FORMATION BY REPRESSING BMP2 TRANSCRIPTION IN OSTEOBLASTS

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Zinc finger Gli proteins mediate hedgehog signaling which is required for osteoblast differentiation. C' terminal truncated Gli3, a proteolytically processed fragment of Gli3, is a predominant and functional form of Gli3 and in general acts as gene transcriptional repressor. Here, we have investigated the role of repressor Gli3 (rGli3) in osteoblasts. We found in osteoblast precursor cells that full-length Gli3 underwent proteolytic processing which resulted in such a truncated rGli3 through the PKA-ubiquitination-proteasome pathway. We determined the effects of rGli3 on osteoblast differentiation and bone formation. *Ex vivo* calvarial organ culture has shown that overexpression of rGli3 significantly decreased proteasome inhibitor-induced calvarial ALP activity and new bone formation over the calvarial surface. These results suggest that rGli3 is an inhibitor of bone formation and this action is likely mediated through BMP2 since we have previously demonstrated that proteasome inhibitors are powerful enhancers of BMP2 expression in osteoblasts. By BMP2 promoter and mRNA assays, we found that rGli3 overexpression markedly inhibited BMP2 transcription in osteoblasts. Interestingly, we also found that rGli3 not only reduced the basal level of BMP2 expression, also antagonized the levels enhanced by Gli2, Smad1, b -catenin/TCF4, all of which have been previously shown to activate BMP2 expression in osteoblasts. To determine the interaction of rGli3 with BMP2 promoter, we performed promoter deletion and mutation studies, and identified three Gli binding elements in the BMP2 promoter responsible for rGli3 repression. These data suggest that rGli3 is a strong transcriptional repressor of BMP2 gene in osteoblasts. Lastly, we determined the role of rGli3 in bone *in vivo*. We generated osteoblast-specific rGli3 transgenic mice using 2.3Col1a1 promoter. m CT and histomorphometric results have shown that bone volume of rGli3 transgenic mice was substantially reduced along with decreased trabecular bone number and thickness and increased trabecular bone separation, compared with those of wild-type mice, suggesting that rGli3 has an inhibitory effect on normal bone mass. Together, our data provide evidence that C' terminal shortened Gli3, the naturally processed product of full-length Gli3, is a strong repressor of osteoblastic bone formation and this function is due to its inhibition of BMP2 expression in osteoblasts.

021

CTHRC1 IS A POSITIVE REGULATOR OF OSTEOBLASTIC BONE FORMATION

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Bone mass is maintained by the balanced action of osteoblastic bone formation and osteoclastic bone resorption. This remodeling process is regulated by many systemic and local factors. Identification of molecules that affect bone mass is important for understanding and potential treatment of bone disease. We identified Collagen triple helix repeat containing 1 (Cthrc1) as a downstream target of bone morphogenetic protein-2 in ATDC5 cells by PCR-based suppression subtractive hybridization, followed by differential hybridization. Cthrc1, a glycosylated protein with a signal sequence, was highly expressed in osteogenic MC3T3-E1 cells by northern blot analyses and in bone tissues by *in situ* hybridization analyses. To investigate the role of Cthrc1 in bone, we generated Cthrc1-null mice and transgenic mice which overexpress Cthrc1 in osteoblasts (Cthrc1 transgenic mice). Micro-CT and bone histomorphometric analyses revealed that Cthrc1-null mice displayed low bone mass by suppressed bone formation, whereas Cthrc1 transgenic mice showed high bone mass by accelerated bone formation. Osteoblast proliferation, assessed by BrdU incorporation, was suppressed in Cthrc1-null mice and stimulated in Cthrc1 transgenic mice, respectively. Furthermore, mRNA expression levels of alkaline phosphatase, Col1a1, and Osteocalcin in primary osteoblasts harvested from calvaria were decreased in Cthrc1-null mice, and increased in Cthrc1 transgenic mice, respectively. Our results indicate that Cthrc1 positively regulates bone formation by stimulating osteoblast proliferation and differentiation.

022

PROTEIN PHOSPHATASE MAGNESIUM-DEPENDENT 1A INHIBITS BMP SIGNALING BY STIMULATING SMAD DEGRADATION INDEPENDENT OF DEPHOSPHORYLATION AT THE CARBOXYL TERMINI

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BMPs induce bone formation through binding to specific receptors. Type I BMP receptors phosphorylate the carboxyl terminal serine residues of Smad1/5/8 to activate downstream signaling of BMPs, such as an Id1 gene expression. Recently, we found that osteoblastic differentiation of myoblasts was induced by an over-expression of a constitutively activated Smad1, in which the carboxyl terminal serine residues have been substituted by aspartic acid residues, suggesting that the Smad signaling pathway is critical for the bone formation induced by BMPs. Protein phosphatase magnesium-dependent 1A (PPM1A) has been shown to suppress BMP activities by dephosphorylating the carboxyl terminal phospho-serine residues as substrates. We report here that PPM1A suppresses BMP signaling via a novel mechanism. In co-transfection experiments, PPM1A inhibited both Id1 promoter activity and ALP activity induced by not only BMP4-treatment but also an over-expression of the constitutively active Smad1. This finding indicated that the inhibitory activity of PPM1A on BMP signaling was independent of dephosphorylation of Smads at the carboxyl termini. Western blotting and immunohistochemical analysis indicated that PPM1A reduced protein levels of Smad1. Similar reduction by PPM1A was observed in wild-type Smad5 and Smad8. PPM1A decreased protein levels of another mutant Smad1, in which a Smurf consensus sequence in the linker region has been destroyed, suggesting that Smurf E3 ubiquitin ligases may not be involved in this process. Treatment cells with a proteasome inhibitor blocked the inhibitory effects of PPM1A on Smad1. In contrast to wild-type, a mutant PPM1A lacking a phosphatase activity did not inhibit BMP signaling and failed to decrease the Smad protein levels. Moreover, knockdown of endogenous PPM1A by siRNA stimulated BMP activity in C2C12 myoblasts. Taken together, these findings suggested that PPM1A suppresses BMP signaling by stimulating Smad degradation independent of dephosphorylation at the carboxyl termini and this inhibitory regulation may play an important role in physiological BMP activities.

023

AP-1 FUNCTION IN MESENCHYMAL CELL FATE DECISION: THE ROLE OF RIBOSOMAL S6 KINASE AND FRA1

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While mice over-expressing the Fos-related protein Fra-1 develop progressive osteosclerosis due to increased osteoblastic differentiation, mice lacking the Fos kinase Rsk2 develop progressive osteopenia with impaired mineralization. These observations suggested a role for Fra-1 phosphorylation by Rsk2 in controlling osteoblast activity. To test this hypothesis, *fra-1* transgenic mice lacking Rsk2 were generated and phenotyped. The mice die prematurely, were severely growth retarded and developed both a severe osteosclerosis and a bone mineralization defect suggesting that Fra-1 and Rsk2 function independently in bone.

However, a total lipodystrophy was observed in the double mutated mice. When analysing the parental strains, a progressive lipodystrophy of the white adipose tissue (WAT) was also observed in *fra1* transgenic but not in Rsk2-deficient mice. The phenotype was more pronounced in females than in males. Brown adipose tissue (BAT) was unaffected. An increased number of immature adipocytes were found in histological sections of the WAT isolated of *fra-1* transgenic mice. In agreement, the expression of markers for fat maturation, *Glut4* and *aP2* were decreased. The phenotype was cell autonomous, since adipogenic potential of primary osteoblasts (POBs) isolated from the calvaria of *fra-1* transgenic mice was impaired. The effect was Fra1-dependent as shown by the constitutive or inducible over-expression of Fra-1 in adipogenic cell line strongly inhibiting adipocyte differentiation.

Osteoblasts and adipocytes differentiate from pluripotent bone marrow mesenchymal stromal cells (MSCs) that can also differentiate into other mesenchymal lineages: myotubes (muscle), chondrocytes (cartilage) or fibroblasts. However, the expression of key regulators of mesemchymal cell fate (i.e. *Runx2, Sox9, MyoD, C/EBP(beta) and C/EBP(delta)* was unchanged, indicative of a normal commitment of the cells. However, the adipogenic transcription factors controlling adipocyte maturation, *C/EBP(alpha)* and *PPAR(gamma2)* , were down-regulated in *fra1*-tg POBs and in adipogenic cell lines over-expressing Fra-1.

Thus, our data demonstrate that increased bone mass can be observed in lean mice. Indeed, in addition to promoting osteoblastogenesis, Fra-1 inhibits adipocyte maturation, this latter being caused by C/EBP(alpha) and PPAR(gamma2) down-regulation. Moreover, we show that Rsk2 is regulating Fra-1 function in fat tissue but not in the bone.

Understanding AP-1 function in mesenchymal cell fate decision may provide valuable insights into mesenchymal tissue regeneration and metabolic diseases.

024

CREB INDUCES BMP2 TRANSCRIPTION IN OSTEOBLASTS AND CREB KNOCKOUT REDUCES BONE MASS IN MICE

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Transcription factor CREB (cAMP-response element binding protein) plays an essential role in osteoblasts to mediate the anabolic signaling of intermittent dosage of PTH in bone. However, the downstream mechanisms of CREB in osteoblasts have to be demonstrated. Here, we have characterized the skeletal phenotype of CREB knockout mice and identified the potential transcriptional target of CREB in osteoblasts. The results of m CT measurement and alizarin red/alcian blue, ALP and von Kossa staining, have shown that global knockout of CREB in mice caused a dwarfism phenotype and a significant reduction of bone volume and mineralization in the embryonic skeleton. Since the global CREB KO mice do not survive after birth, we have generated the osteoblast-specific CREB KO mice (CREBfl;2.3ColCre) and examined their skeletal phenotype. m CT results have shown that bone volume of two-month-old CREBfl;2.3ColCre KO mice substantially reduced, along with decreased trabecular number and thickness and increased trabecular separation, compared with that of control mice. These results suggest that CREB is critical for both normal skeletal development and postnatal bone mass. Interestingly, we found that expression of BMP2, an important factor for osteoblast differentiation and bone formation, was reduced in the bones of CREB KO mice. Since there exists a similar osteopenic phenotype between CREB KO CREBfl;2.3ColCre) and BMP2 KO (BMP2fl;3.6ColCre) mice, and the BMP2 promoter contains multiple cAMP response elements (CRE), we hypothesized that the function of CREB in bone is mediated at least in part through BMP2. In cell culture, we found that both CREB and PTH stimulated BMP2 gene expression in osteoblasts. We also found that pharmacological manipulation of CREB phosphorylation by cAMP/PKA activator IBMX or inhibitor KT5720 affected CREB transactivation of the BMP2 expression. Furthermore, through promoter mutation studies, we demonstrated that CREB transactivated BMP2 gene by directly interacting with a specific CRE in the BMP2 promoter. Lastly, we have shown that overexpression of CREB promoted osteoblast differentiation and this action was blocked by addition of noggin in the cultures. Together, these results suggest that CREB plays an important role in osteogenesis embryonically and postnatally, and this function is mediated by up-regulation of BMP2 transcription in osteoblasts.

025

OSTEOBLAST IL-33 MRNA EXPRESSION IS REGULATED BY PTH, AND IL-33 TREATMENT CAUSES BOTH INCREASED OSTEOBLASTIC MATRIX MINERALISATION AND REDUCED OSTEOCLAST FORMATION *IN VITRO*

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IL-33 is a Th2 stimulating pro-inflammatory cytokine related to IL-1 and IL-18, its actions mediated by receptor ST2L. We have previously found that IL-33, like IL-18, indirectly inhibits osteoclast formation via T lymphocytes but does not directly affect osteoclast formation from RANKL-stimulated bone marrow macrophages (BMM).

In mouse bone sections, anti-IL-33 antibody clearly immunostained osteoblasts and chondrocytes and some osteoclasts but not osteocytes or most bone marrow cells. DNA microarray array studies also showed ST2L mRNA expression in matured osteoblastic Kusa 4b10 cells is increased by PTH treatment, observations confirmed by further RT-PCR analysis. PTH treatment also increased IL-33 mRNA levels in osteoblasts. Long term osteoblastic cultures treated with ascorbate (which increases osteoblastic differentiation) also increased IL-33 mRNA levels. With long term pro-adipogenic treatment (dexamethasone, insulin and IBMX) of immature Kusa 4b10 cells IL-33 mRNA levels increased but ST2L mRNA levels decreased.

Since osteoblasts express ST2L we investigated IL-33 action on osteoblasts to identify possible autocrine actions. IL-33 promoted matrix mineralisation by primary osteoblasts. Furthermore, in long term ascorbate stimulated primary osteoblasts in which expression of osteocytic features are apparent (e.g. sclerostin and DMP-1 expression), IL-33 reduced sclerostin mRNA levels after 6 and 24 hours of treatment, although other PTH regulated genes in these cells, such as ephrin B2 were not affected.

We also investigated IL-33 effects on osteoblastic support of osteoclastogenesis. Osteoclast formation from BMM stimulated by 1,25 dihydroxyvitamin D3-treated Kusa O pre-osteoblastic cells was blocked in the presence of IL-33 (20ng/ml), an action ablated by anti-GM-CSF antibody. GM-CSF mRNA was strongly upregulated by IL-33 treatment, as indeed was RANKL mRNA. However, Kusa O/BMM co-cultures treated with IL-33 and anti-GM-CSF antibody (without other stimulus) induced osteoclast formation only weakly.

Thus we have found evidence that IL-33 stimulates osteoblastic function while indirectly, through two separate mechanisms, inhibiting osteoclast formation. IL-33 thus may play a role in maintaining bone mass, perhaps participating in the anabolic actions of PTH.

026

A DAIRY-BASED PROTEIN, CALCIUM AND VITAMIN D SUPPLEMENT PRESERVES TRABECULAR BONE AND REDUCES FALLS IN AGED CARE RESIDENTS

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Aged care residents are at higher risk of falls and fractures than elderly living in the community. Nutrient deficiencies may increase falls and fracture risk by contributing to bone loss and body sway and reducing muscle mass and strength. We aimed to determine if a dairy-based protein, calcium and vitamin D supplement incorporated into foods would reduce falls and fracture risk in elderly aged care residents. We studied 1200 residents from 13 control and 7 intervention hostels over 2 years, with the first year being observation only in all residents and the 2nd period involving observation (controls) or intervention with approximately 5g protein, 300mg calcium and 500 IU vitamin D3. 71 females provided blood samples, 65 had BMD assessed using densitometry and 44 underwent bone structure assessment at the distal radius and tibia using high-resolution micro-pQCT. Continuous data was analysed using repeated measures ANOVA, comparison of falls before and after treatment using Wilcoxon Signed Rank Test and Chi Squared distribution was used to determine the effect of intervention on changes in falls frequency. Compared to controls, supplementation reduced the decline in vitamin D (-22% ± 10% v -61% ± 12%, $P < 0.05$), while PTH was lowered by treatment (-16% ± 5%, $P < 0.01$), but remained unaltered in controls (5% ± 6%). Loss of trabecular BMD (-5 ± 3%, NS) and bone volume to total volume (-3 ± 3%) at the radius was prevented compared to controls who lost bone (both -10 ± 3%), $P < 0.05$). Femoral neck BMD was maintained in supplemented residents, but 4% lower in controls ($p < 0.1$). In those who fell before intervention, falls rates were lower during supplementation (1.3 per person) v prior (2.2 per person, $p < 0.01$) with the benefits most evident in those with recent falls histories ($p < 0.01$).

Nutritional supplementation prevents loss of FN BMD and trabecular bone and reduces the incidence of falls rates, in particular in those with a recent history of falls.

027

MECHANICAL STRESS ENHANCES OSTEOBLAST DIFFERENTIATION VIA CANONICAL WNT PATHWAY

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Mechanical stress to bone plays an important role in the maintenance of bone homeostasis. Reduced mechanical loading by prolonged bed rest, immobilization or microgravity in space has been shown to cause a marked loss of bone. However, the molecular mechanism of mechanical stress-induced bone formation is not fully understood. In the present study, we demonstrated that mechanical loading reduces expression of a Wnt inhibitor via induction of interleukin (IL)-11 to stimulate osteoblast differentiation. Using tail-suspended mice in vivo, mechanical unloading suppressed and re-loading enhanced IL-11 gene expression. Mechanical stress to cultured osteoblasts by fluid shear stress (FSS) in vitro also enhanced the expression of IL-11 with stimulation of osteoblastogenesis and inhibition of adipogenesis. Addition of anti-IL-11 blocking antibody abrogated the enhancement of osteoblastogenesis and suppression of adipogenesis by FSS. To investigate the mechanism whereby IL-11 stimulated osteoblast differentiation, effect of mechanical stress on Wnt/beta-catenin signaling was examined using TopFlash reporter assay. FSS to osteoblasts enhanced Tcf/Lef transcription activity, and IL-11 siRNA markedly suppressed the stimulation of Wnt/beta-catenin signaling by FSS. The enhancement of Wnt/beta-catenin signaling by mechanical stress was mediated by a suppression of a Wnt/beta-catenin signal inhibitor, Dkk2, and in vivo mechanical unloading strongly induced and reloading suppressed Dkk2 expression. Dkk2 expression was also suppressed by FSS in vitro, and knockdown of IL-11 by siRNA reduced expression of Dkk2 induced by FSS. These observations demonstrate that mechanical stress stimulates IL-11 gene transcription, and that increased IL-11 enhances osteoblastogenesis and suppresses adipogenesis via stimulation of Wnt/beta-catenin signaling at least in part by a suppression of Dkk2 expression.

028

OSTEOBLAST SPECIFIC Y1 DELETION ENHANCES BONE FORMATION

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Neuropeptide Y (NPY) has been shown to play a critical role in the regulation of bone metabolism by signaling via Y1 and Y2 receptors. Centrally, hypothalamic Y2 but not Y1 receptors have been shown to be important for the action of NPY on bone formation and osteoblast activity. However, the peripheral mechanism remains unknown. In-situ hybridisation on femur sections reveals the presence of Y1 but not Y2 receptor mRNA in osteoblasts, consistent with a direct role for the Y1 receptor on bone cells.

To investigate the role of the Y1 receptor on osteoblastic cells, we generated mice with selective deletion of the Y1 receptor in osteoblasts by crossing Y1lox/lox mice with 2.3ColCre and 3.6ColCre mice expressing Cre specifically in osteoblasts utilising different regions of the α1(I)-collagen promotor. The 3.6ColCre line expresses Cre early during osteogenic differentiation whilst in the 2.3ColCre line, Cre expression is restricted to maturing osteoblasts.

In 16 week old male mice body weight was unaltered in both lines of ColCre;Y1lox/lox mice when compared to their Y1lox/lox littermates. Importantly, whole body bone mineral density was increased in both 2.3ColCre;Y1lox/lox ($p=0.05$) and 3.6ColCre;Y1lox/lox mice ($p=0.05$). Osteoblast-specific Y1 receptor deletion also resulted in a marked increase in femoral cancellous bone volume (2.3ColCre;Y1lox/lox 16.2 ± 1.6, 3.6ColCre;Y1lox/lox 16.6 ± 1.2, compared to Y1lox/lox 12.0 ± 1.1 %; $p=0.05$ and $p=0.05$ respectively). This increase in bone volume was associated with an increase in mineral apposition rate (2.3ColCre;Y1lox/lox 2.31 ± 0.06, 3.6ColCre;Y1lox/lox 2.31 ± 0.06, compared to Y1lox/lox 2.01 ± 0.07 mm/day; $p=0.004$ and $p=0.005$ respectively). No significant differences were observed in osteoclast number or osteoclast surface area between groups suggesting that bone resorption has not been

affected.
Together these data demonstrate a direct role for the Y1 receptor on osteoblasts in the regulation of osteoblast activity and bone formation in vivo. Understanding the action of NPY on osteoblasts to regulate bone metabolism will have powerful therapeutic implications in diseases such as osteoporosis.

029

CNTF REGULATES MINERALISATION AND PLAYS CRITICAL SEX-SPECIFIC ROLES IN BONE FORMATION

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The family of cytokines that signal through gp130 including IL-6, IL-11, CT-1 and LIF play a critical role in bone cell biology. One member which has not been investigated in bone is Ciliary Neurotrophic Factor (CNTF), which signals by binding to CNTFR then forming a complex with gp130 and LIFR. The same complex is utilised by Neuropoietin (NP) and cardiotrophin-like-cytokine (CLC). CNTFR has been described in osteoblast-like cells suggesting a role for these cytokines in bone metabolism, but no specific function has yet been described. Using real-time PCR, we confirmed expression of CNTFR in primary calvarial osteoblasts, which was upregulated (60 fold) over the course of osteoblast differentiation. Immunohistochemistry of normal mouse bone detected CNTF in osteoblasts and osteoclasts, and CNTF in osteoblasts suggesting a paracrine role for CNTF in bone metabolism. Treatment of osteoblasts differentiated for 14 days with CNTF, NP or CLC blocked further mineralisation, such that, by day 21 there was a significantly lower level of mineralisation in the wells containing treated osteoblasts. Furthermore, mRNA levels of osterix and sclerostin was significantly downregulated 6 hours after CNTF or NP treatment.
To investigate this further, we analysed the bones of adult CNTF null mice using histomorphometry and pQCT. In 12 week old male CNTF KO mice femoral length, cortical thickness and periosteal circumference were significantly reduced, but there was no significant change in trabecular bone mineral density (Tb.BMD) or tibial trabecular bone volume (BV/TV). In contrast, female CNTF null mice femora were of normal length and width, femoral BMD was significantly increased (by 30%), and tibial BV/TV, trabecular number and trabecular thickness were all significantly increased. The increase in trabecular bone in CNTF null mice appeared to relate to a significant (20%) increase in osteoblast number as well as a significant (30%) increase in mineral apposition rate. Taken together, this data identifies two novel and sex specific roles for CNTF: in female mice as a paracrine inhibitor of trabecular bone formation, and in male mice as a stimulus of cortical apposition.

035

SKELETAL STEM CELLS AND THE MICROVASCULAR SYSTEM OF TISSUE SPECIFIC PROGENITORS

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The bone marrow has long been know to comprise a population of multipotent cells, originally defined as "osteogenic" or "stromal" stem cells, and later renamed "mesenchymal stem cells", which have now been identified as residing abluminally to endothelial cells in bone marrow sinusoids. These cells have been shown to be able to regenerate, besides bone and other skeletal tissues, a layer of subendothelial cells in sinusoids formed de novo in heterotopic ossicles generated by transplantation of purified populations of skeletal stem cells. While providing the long missing evidence for self-renewal of skeletal stem cells, these data have suggested that skeletal stem cells can specifically function in guiding the formation and remodeling of nascent blood vessels in vivo, a function shared with a broad class of ubiquitous subendothelial cells known as pericytes or mural cells. Indeed, novel in vivo transplantation assays in which the vasculogenic function of skeletal stem cells can be probed in isolation (i.e., with no interference of simultaneous bone formation processes), and in which skeletal stem cells are co-transplanted with endothelial cells, reveal the unique ability of skeletal stem cells to direct the formation of a network of functional, stable, human blood vessels in vivo, highlighting what could indeed represent a (or the) crucial physiological function played by skeletal stem cells in vivo. In vivo gene knockdown experiments combined with xenotransplantation assays are elucidating the role of specific genes, such as angiopoietin-1 and MCAM, in the vasculogenic function of skeletal stem cells, and are at the same time revealing unexpected, "vasculocentric" mechanisms of regulation of the differentiation of

subendothelial cells into differentiated phenotypes. Meanwhile, it is becoming clear that it is the strategic positioning of skeletal stem cells at the abluminal side of bone marrow sinusoids that puts them at center stage in the regulation of cell traffic across the sinusoids. This has major implications for the role that skeletal stem cells play in maintaining a niche for hematopoietic stem cells, but also in providing a homing site for blood borne, hematopoietic and non-hematopoietic, cancer cells. Finally, the isolation of subendothelial cells from organs and tissues other than bone marrow is revealing a complex system of homologous and yet different, tissue specific progenitors of mesoderm-derived tissues, and is finally opening glimpses on the specific and yet simple developmental processes whereby postnatal progenitors are established in postnatal bone and other tissues.

036

ARTERIOSCLEROSIS AND BONE DISEASE

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Tremendous unmet needs exist in musculoskeletal medicine. Osteoporosis and osteoarthritis are recognized as common and important, but other serious skeletal disorders increasingly afflict our world. In the setting of type 2 diabetes (T2DM), lower-extremity (LE) musculoskeletal disease is prevalent, costly, and exceedingly difficult to manage, with fracture, arthropathy, ischemia, ulcer, infection, and amputation commonly confronting patients and clinicians. Arteriosclerosis is a chronic vascular disease common in T2DM, characterized by abnormal thickening and hardening of arterial walls with loss of vessel compliance. Atherosclerosis, medial calcific sclerosis, and arteriolosclerosis are the three pathologic types of arteriosclerosis. With advanced age, diabetes, hypertension, and uremia, conduit arteries become arteriosclerotic, losing the elasticity necessary to ensure smooth distal tissue perfusion with efficiency that minimizes cardiac workload. This Windkessel physiology is impaired by not only changes in vascular geometric properties, but also by changes in vascular material properties -- biochemical alterations that include fibrosis, chemical crosslinking, and matrix mineralization. With vessel stiffening, systolic barotrauma and diastolic underperfusion are problematic in tissues and organs. Arteriosclerosis is well-known to increase risk for stroke, myocardial infarction, and heart failure. However, medial calcific sclerosis has emerged as a particularly important contributor to LE amputation risk in T2DM. Indeed, tibial artery calcification scores predict LE amputation risk better than ankle-brachial systolic pressure indices commonly used to assess risk for critical limb ischemia. A better understanding of signaling pathways that control arterial matrix fibrosis and calcification will lead to new strategies for diminishing musculoskeletal disease burden. Recent data from labs worldwide implicate osteogenic Wnt cascades as important in vascular disease, including diabetic arteriosclerosis. Paracrine Wnt/Dkk signals control arterial calcification by regulating osteogenic lineage allocation of vascular mesenchymal progenitors. Peroxide- and oxylipid- dependent cues -- initiated by TNF-alpha and oxidase activities -- activate this osteogenic Wnt injury response. With progression to renal insufficiency, a common complication of diabetes, hyperphosphatemia accelerates arterial calcium accrual. Low turnover bone disease accentuates vascular calcification via unclear mechanisms. Thus, strategies that reduce vascular peroxide and oxylipid accumulation, inhibit arterial osteogenic Wnt signaling, and maintain normal phosphate homeostasis are predicted to mitigate arteriosclerotic disease and reduce LE amputation risk.

037

A NOVEL SMALL THIENOINDAZOLE-DERIVATIVE COMPOUND INDUCES CHONDROGENIC DIFFERENTIATION WITHOUT PROMOTING HYPERTROPHY THROUGH PRODUCTION OF RUNX1

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Aiming at regeneration of permanent cartilage like joint cartilage, we have screened natural and synthetic compound libraries using stable lines of ATDC5 cells expressing green fluorescent protein (GFP) under the control of type II collagen promoter fused with four repeats of a Sox9 enhancer (COL2-GFP) as a monitoring system for chondrogenic differentiation. We found that a novel compound, a thienoindazole-derivative compound T-198946 (TM), most strongly induced the GFP fluorescence as early as after 48 h of treatment. TM was confirmed to enhance chondrogenic

differentiation but inhibit the further hypertrophic differentiation in the cultures of immature mesenchymal C3H10T1/2 cells, determined by real-time RT-PCR analysis for the chondrogenic markers. To clarify its transcriptional targets and signal transduction mechanism, we screened for the target molecules of TM by the microarray analysis and revealed that Runx1 was most strongly induced by TM among 581 up-regulated genes including Sox5 and Sox6. Luciferase-reporter analyses using deletion, mutagenesis, and tandem-repeat of the COL2 promoter identified the core responsive element of Runx1 in the COL2 promoter to be between the -293 and -288 bp region containing a putative Runx-binding motif. The specific binding of Runx1 to this region was confirmed by EMSA and ChIP assays. For functional analyses, we performed adenoviral overexpression of the gene or the small interfering RNA in C3H10T1/2 cells. Although chondrogenic differentiation was enhanced by the Runx1 overexpression alone, it was further enhanced by co-transduction with Sox5, 6, and 9 (the Sox trio), without promoting the hypertrophy, similar to the effect of TM treatment. Gene-silencing of Runx1, Sox5/6, or Sox9 suppressed the TM effect on chondrogenic differentiation. Immunohistochemistry revealed that Runx1 and the Sox trio were co-localized in the proliferative and pre-hypertrophic chondrocytes of the mouse growth plate, and their physical interaction was confirmed by immunoprecipitation and two-hybrid analysis. Finally, full-thickness defects of mouse knee cartilage was completely filled with cartilaginous tissue after transplantation of cell-sheets of TM-treated chondrocytes, while the control cell-sheets did not. A novel small compound TM induces chondrogenic differentiation without promoting hypertrophy, through production of Runx1 that cooperatively functions with the Sox trio. TM will herald a new era of regenerative medicine of permanent cartilage, thus providing an epochal treatment of osteoarthritis.

038

OSTEOMACS ARE CRITICAL FOR OPTIMAL INTRAMEMBRANOUS BONE FORMATION IN A TIBIAL DEFECT MODEL OF BONE HEALING.

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Osteal tissues contain a resident population of macrophages (OsteoMacs) that regulate osteoblast mineralisation *in vitro*. OsteoMacs form a canopy structure covering osteoblast bone forming surfaces (ObS) suggesting OsteoMacs regulate bone anabolic responses. Using the Mafia (Macrophage-Fas-Induced Apoptosis) transgenic mouse, in which the *csf1r* promoter drives the expression of AP20187 (1) ligand-inducible Fas-based suicide gene in macrophage/myeloid cells, we demonstrated that OsteoMacs are required for maintenance of ObS at sites of bone modelling. To definitively examine OsteoMacs contribution to *in vivo* bone formation a tibial defect model was employed in which the defect site is filled with woven bone via intramembranous ossification 7 days post surgery. Immunohistochemistry demonstrated that $F4/80^+$ OsteoMacs accumulated within the defect site, forming the canopy structure covering collagen type I $(CTI)^+$ osteoblasts on woven bone surfaces. Osteotomies were performed in Mafia mice and a single injection of ligand or vehicle administered intra-defect at surgery. Healing was examined 7 days post surgery. Flow cytometry confirmed a 46 ± 7% decrease in bone marrow $F4/80^+$ macrophages in the contralateral limb of ligand treated mice. In ligand treated animals there was a striking reduction in the number of $CT1^+$ osteoblasts and $F4/80^+$ OsteoMac canopy within the defect. Quantitative immunohistochemical analysis of intra-defect CTI^+ matrix area demonstrated a significant reduction ($p= 0.03$) in bone formation in ligand (20 ± 6%) compared to vehicle (44 ± 5%) treated mice. Osteoclasts express *csf1r* and therefore may be susceptible to apoptosis in ligand treated Mafia mice. To rule out that the decreased bone formation was indirectly due to inhibition of osteoclasts, osteotomies were performed in C57/Bl6 mice that were treated with OPG (1mg/kg, initial intra-defect administration and subsequently subcutaneous injection every second day) to specifically inhibit osteoclasts. Immunohistochemical analysis of OPG treated animals demonstrated minimal change in intra-defect CTI^+ woven bone deposition or defect healing. Distribution of $F4/80^+$ OsteoMacs within the defect was unaffected by OPG treatment. These observations support that OsteoMacs, and not osteoclasts, play a critical role in driving and maintaining osteoblast function at sites of intramembranous bone deposition during growth and bone healing. Therefore OsteoMacs are novel participants in bone formation *in vivo*.

(1) www.ariad.com/regulationkits

039

REGULATION OF ADIPOGENESIS BY ZFP467, A NOVEL ZINC-FINGER PROTEIN

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Broadening our current understanding of the molecular mechanisms of PTH induced bone formation will lead to the development of more effective treatments for bone disorders. Investigating gene regulation in differentiated mouse marrow stromal cells (Kusa 4b10) treated with PTH (1-34) and probed onto an Affymetrix whole mouse genome microarray, has identified zinc-finger protein 467 (Zfp467) as a potential regulator of adipogenesis. Zfp467 belongs to the Krüppel-like family of transcription factors that bind to GC-rich DNA elements to regulate cellular functions such as proliferation, differentiation and cell fate determination. Zfp467 is also known to be regulated by oncostatin M (a gp130-family cytokine) and can transactivate promoters responsive to STATs. This study focused on the role of Zfp467 in stromal cell differentiation. Zfp467 mRNA was significantly repressed after treatment of both Kusa 4b10 cells and primary mouse calvarial osteoblasts with either PTH or gp130 cytokines (including OSM, LIF, CT-1 and IL-11) known to influence osteoblast activity. To delineate the role of Zfp467 in osteoblast function, it was overexpressed using a retroviral system in Kusa 4b10 cells. Overexpression significantly decreased the rate of mineralisation and increased adipocyte formation in mineralising media. The reverse effect was observed when antisense Zfp467 was introduced. These differences in mineralisation correlated with real-time PCR analyses for osteoblast and adipocyte markers. Overexpression of Zfp467 reduced mRNA levels relative to control for the osteoblast markers osterix (7 fold at 11d), ALP (40 fold at 13d) and osteocalcin (400 fold at 15d) and elevated levels of the adipogenic markers C/EBPα (6 fold at 2d), adiponectin (500 fold at 4d) and resistin (200 fold at 9d). When Kusa 4b10 cells overexpressing Zfp467 were differentiated in adipogenic media, adipogenesis was accelerated, and concomitant increases in the steady state levels of mRNAs for PPARγ, C/EBPα, adiponectin and resistin were noted. Thus Zfp467 contributes to stromal cell differentiation in favour of adipogenesis rather than osteoblastogenesis. Future studies aim to determine the molecular mechanisms by which Zfp467 diverts differentiation preferentially to adipocytes.

040

CALCITONIN ATTENUATES THE ANABOLIC EFFECT OF PTH IN YOUNG RATS BY RAPID UPREGULATION OF SCLEROSTIN EXPRESSION

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Having found that the anabolic effect of PTH in weanling rats was inhibited by co-treatment with a low dose of salmon calcitonin (sCT) that transiently inhibited osteoclast function, we sought to identify mediators of this effect. Gene expression was examined in metaphyseal bone of 3 week old female rats treated with vehicle, 0.5μg/kg sCT, 30μg/kg hPTH(1-34) or co-administered sCT and hPTH(1-34). Bones were collected at 1.5, 4 and 6 h after treatment. Femoral metaphyseal mRNA was analysed by quantitative real time PCR (qRTPCR) and tibial sections prepared for immunohistochemistry. Several gene products affected by PTH, including RANKL, IL-6 and ephrinB2, were not modified by simultaneous sCT treatment. In contrast, the PTH-induced reduction in mRNA for the osteocyte-derived bone formation inhibitor sclerostin was diminished by sCT co-administration at all times. Furthermore, sCT alone increased sclerostin mRNA > 2.5-fold at 1.5, 4 and 6 hrs. This was confirmed by immunohistochemistry in tibiae, where the percentage of sclerostin positive osteocytes in cortical bone was significantly increased at 4 hrs following sCT administration (vehicle: 26 ±4%; sCT: 43 ±3%, $p<0.01$). Also at 4 hrs, sCT reduced mRNA for other osteocytic gene products including MEPE (-1.4 ±0.06 fold, $p<0.001$) and DMP-1 (-1.9 ±0.06 fold, $p<0.001$).

The only known target of CT in bone is the osteoclast, but we did not detect sclerostin production by osteoclasts, and sCT did not affect osteoclast production of BMP-2, the only sclerostin production stimulus. There is no evidence of CT receptors within the osteoblast lineage, and receptor autoradiography and RT-PCR confirmed this in calvarial osteoblasts from normal and DMP-1Cre.GFP mice. Furthermore production of sclerostin by these osteocyte-like cells was unaffected by sCT, CGRP or amylin treatment. These data indicate that calcitonin promotes sclerostin production, and influences production of other osteocytic genes, and suggest this is a pathway by which calcitonin might modify the anabolic effect of PTH in the young, growing rat. This effect of calcitonin on sclerostin production by osteocytes

may be the result of an indirect action, either through the osteoclast or through some non-skeletal site of calcitonin action, which could include the brain.

041

EPHRIN B2 - EPHB4 EFFECTS IN OSTEOBLAST DIFFERENTIATION: ROLE OF RHOA SIGNALING.

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Members of the ephrin and Eph family are local mediators of cell function through largely contact-dependent processes in development and maturity. Production of ephrinB2 mRNA and protein by osteoblasts are increased by PTH and PTHrP. In differentiating murine preosteoblasts (Kusa 4b10) and calvarial osteoblasts, antagonists of the ephrinB2/EphB4 interaction were used to study mineralization and the expression of several osteoblast genes involved late in osteoblast differentiation. Of the two classes of receptor antagonist, one was the peptide, TNYLFSPNGPIARAW, a specific antagonist of ephrinB2 interaction with EphB4. The second was the recombinant extracellular domain of EphB4 (sEphB4), which inhibits both forward and reverse signaling between ephrinB2 and EphB4. Each of these receptor antagonists inhibited mineralization by the Kusa 4b10 mouse stromal cells in a dose-dependent manner. Furthermore in Kusa 4b10 cells at a late stage of differentiation, both sEphB4 and TNYL inhibited the expression of mRNA for a number of genes associated with osteoblast differentiation including osteocalcin, bone sialoprotein, PTH1R, sclerostin, DMP-1 and Bril. In mouse calvarial osteoblasts differentiated over 7 days in conditions that result in several hundred-fold increase in expression of osteocyte markers, both sEphB4 and TNYL decreased expression of mRNA for osteocalcin, sclerostin, DMP-1 and MEPE. Since forward signalling through EphB4 increases osteoblast differentiation through inactivation of the small GTPase, RhoA, we treated differentiating Kusa 4b10 and calvarial osteoblast cells with RhoA inhibitors (H1152, 0.1-1 m M and Y27632 2-10 μM). This resulted in greatly increased bone nodule formation *in vitro* and increased expression of the same mRNAs that were inhibited by blockade of ephrin B2-EphB4 receptor interaction (sclerostin 18-fold, DMP-1 6 fold, Bril 4 fold and BSP 3 fold). Immunostaining shows the production of both ephrin B2 and EphB4 to be confined to clumps of osteoblasts predominantly in mature lamellar bone, suggesting involvement predominantly in remodelling. These findings are consistent with ephrinB2/EphB4 signalling within the osteoblast lineage having a paracrine role in osteoblast differentiation, in particular affecting expression of genes relatively late in osteoblast differentiation. Such regulation under the influence of locally produced factors such as PTHrP and prostaglandin E might contribute to control of the filling of resorbed spaces at bone remodeling sites.

042

ROLE OF NEUROPEPTIDE Y IN CONTROL OF BONE FORMATION IN LEPTIN DEFICIENT MICE.

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Leptin and neuropeptide Y (NPY) dependent pathways from the hypothalamus have marked control over osteoblast activity. In cancellous bone, leptin-deficit (ob/ob) mice have increased anabolism, via β-adrenergic signaling. However, cortical bone mass is reduced, despite their obesity, although the mechanism for this effect is unknown. Hypothalamic NPY has a generalized anti-osteogenic effect, also reducing cortical bone mass, while significantly increasing weight (1). Moreover, in the hypothalamus, leptin and NPY are inversely regulated, with increased NPY expression in ob/ob mice. We hypothesize that the reduced cortical bone mass of ob/ob mice is the result of NPY mediated processes.

Femoral osteoblast activity of 16 week, male ob/ob and NPY deficient ob/ob mice (NPY/ob) was examined in both cortical and cancellous bone.

Body weight was significantly reduced in NPY/ob compared to ob/ob mice (45.3 ±1.2 vs 50.2 ±2.0 g, $p<0.05$), although femoral length was unchanged (16.0 ±0.1 vs 15.5 ±0.2 mm). Bone formation was greater in NPY/ob mice at the mid-shaft on both periosteal (1.36 ±0.10 vs 0.91 ±0.10 μm/d, $p<0.01$) and endosteal surfaces (1.39 ±0.18 vs 0.35 ±0.22 μm/d, $p<0.005$). Despite the magnitude of the periosteal effect (1.5-fold), mid femoral cortical dimensions were not different between genotypes, indicating a late-onset effect.

In cancellous bone, there were no additive effects of leptin and NPY deficiency in cancellous bone volume (14.1 ±0.9 vs 14.7 ±1.5 %) and mineral apposition rate (1.66 ±0.06 vs 1.67 ±0.07 μm/d), consistent with previous reports (2).

These data indicate that NPY plays an important role in modulation of cortical bone anabolism in ob/ob mice, and indicates for the first time a putative mechanism behind the opposing cortical and cancellous responses in this model. Given the relative abundance of cortical bone, the most prominent skeletal phenotype in ob/ob mice appears to be an NPY-mediated inhibition of cortical bone formation.

(1) Baldock PA et. al. (2005) J Bone Miner Res. 20:1851-1857
(2) Baldock PA et.al (2006) J Bone Miner Res. 21:1600-1607.

043

MURINE ONCOSTATIN M (MOSM) REGULATES OSETOBLASTIC GENES THROUGH A NOVEL RECEPTOR.

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Oncostatin M stimulates osteoclast formation by enhancing osteoblastic expression of RANKL. Murine (m)OSM signals by binding to gp130 then recruiting OSM receptor (OSMR) prior to activating STAT5 phosphorylation. Human (h) OSM may also recruit LIF receptor (LIFR), which activates STAT3 phosphorylation. We assessed the role of OSMR signalling in bone by studying the phenotype and isolated cells of OSMR knockout mice.

Adult $OSMR^{-/-}$ mice displayed a mild osteopetrotic phenotype caused by reduced osteoclast formation. $OSMR^{-/-}$ bone marrow cells (BMC) stimulated with RANKL and M-CSF *ex vivo* formed normal numbers of OC, normal osteoclastogenic potential of the OC precursor population. In co-cultures of WT or $OSMR^{-/-}$ BMC, the osteoclastogenic response to mOSM was completely ablated in the presence of $OSMR^{-/-}$ pOBs and the mOSM-induced increase in RANKL mRNA levels was ablated in $OSMR^{-/-}$ primary osteoblasts (pOBs), indicating that mOSM stimulates osteoclast formation exclusively through OSMR.

Adult $OSMR^{-/-}$ mice also displayed impaired bone formation *in vivo* and impaired mineralisation by cultured pOBs. In concordance with this, mOSM strongly stimulated mineralisation *in vitro* and promoted bone formation when administered over calvariae of wild type (WT) mice. WT pOBs treated with mOSM demonstrated a rapid increase in C/EBP δ and a reduction in SOST mRNA, both of which would promote osteoblast-mediated bone formation. Surprisingly qRT-PCR analysis of these genes in response to mOSM in $OSMR^{-/-}$ pOBs showed an identical response.

To determine whether the effects of mOSM in the absence of OSMR could be mediated by LIFR we used a LIF antagonist (LA). As expected, LA blocked the effects of hOSM on RANKL and SOST. LA could not block the RANKL response to mOSM in WT pOBs confirming that this effect is mediated by OSMR. Surprisingly the SOST response to mOSM in WT pOBs was not blocked by LA or by deletion of OSMR. Western blot analysis confirmed that mOSM did not enhance STAT3 or STAT5 phosphorylation in $OSMR^{-/-}$ cells. Taken together this suggests that mOSM modifies expression of genes involved in osteoblast differentiation through an alternate receptor to OSMR and LIFR.

044

OSTEOMACS MAINTAIN THE ENDOSTEAL HEMATOPOIETIC STEM CELL NICHE AND PARTICIPATE IN MOBILIZATION.

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OsteoMacs are tissue macrophages that reside within bone lining tissues. OsteoMacs enhance osteoblast function in vitro and maintain osteoblast bone forming surface in vivo. Given the endosteum is a preferred niche for hematopoietic

stem cells (HSC), we investigated whether OsteoMacs participate in HSC mobilization induced by granulocyte colony-stimulating factor (G-CSF). Immunohistochemistry (IHC) using the F4/80 antibody demonstrated that mobilization induced by G-CSF resulted in a sharp reduction in the number of F4/80+ OsteoMacs within the endosteal niche from days 2—6 post initiation of G-CSF administration. At day 2, clustering of F4/80+ cells in the central marrow and in particular around endothelial sinuses was evident. In contract, localization and maintenance of OsteoMacs within the periosteum was unaffected by G-CSF administration. Flow cytometry demonstrated increased numbers of F4/80+ macrophages in blood from days 1-6, indicating egress of macrophages from bone marrow to blood. Static and dynamic histomorphemtry and IHC for osteocalcin expression demonstrated a concomitant significant reduction in endosteal osteoblast number and function as well as osteoid. Notably, the kinetics of OsteoMac loss strikingly parallels, if not precedes, that of endosteal osteoblasts loss and mobilization of HSC to blood. In contrast, osteoclast numbers were not significantly altered by G-CSF administration and treatment with the bisphosphonate zoledronate increased HSC mobilization in response to G-CSF. F4/80+ macrophages repopulation of bone marrow and endosteal surfaces initiated at day 8, 2 days after cessation of G-CSF administration, and was quickly followed at day 10 by recovery of OsteoMac canopy covering cubiodal osteoblast-like cells. Prior experimentation has indicated that mobilization induced by G-CSF acts through an intermediary G-CSF receptor expressing myeloid population. Finally, we demonstrate a pivotal role of macrophages in maintaining endosteal HSC niches in transgenic MAFIA mice in which macrophages can be specifically deleted in vivo using AP20187. At day 5 following macrophages deletion, we observed a collapse in the number of osteoblasts lining the endosteum and robust mobilization of HSC in peripheral blood and spleen. Given macrophages can express the G-CSF receptor and the observations reported here, we propose that G-CSF induces HSC mobilization through OsteoMac-directed collapse of the endosteal HSC niches.

045

ON THE ORIGINS OF FRACTURE RESISTANCE AND ITS BIOLOGICAL DEGRADATION IN HUMAN BONE

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The age-related deterioration of both the fracture properties and the architecture of bone, coupled with increased life expectancy, are responsible for increasing incidences of bone fracture in the elderly segment of the population. In order to develop effective treatments, an understanding of the mechanisms underlying the structural integrity of bone, in particular its fracture resistance, is essential. The origins of the toughness of human cortical bone are described in terms of the contributing micro-mechanisms and their characteristic length scales in relation to the hierarchical structure of these mineralized tissues. It is shown that although structure at the nanoscale is important, it is microstructural features at the scale of one to hundreds of microns (*e.g.*, the Haversian systems present in the cortical bone of mammals) that are most important in determining its fracture properties. We specifically find that the origins of fracture resistance in human cortical bone are extrinsic, *i.e.*, associated primarily with crack growth, and are related to such toughening mechanisms as gross crack deflection/twist and crack bridging, both processes that are induced by preferential microcracking (primarily at the cement lines). In particular, our results, in terms of full nonlinear elastic crack-resistance curve measurements, show that human cortical bone is actually much tougher in the transverse orientation than has been previously thought.

In this context, realistic short-crack measurements of both crack initiation and growth toughnesses performed on human and animal bone are used to evaluate the effects of aging and certain therapeutic treatments (*e.g.*, steroids and bisphosphonates). These measurements are combined with structure characterization using UV Raman spectroscopy, small-angle x-ray scattering and transmission electron microcopy and imaging studies involving two-dimensional *in situ* fracture tests performed in an environmental scanning electron microscope and three-dimensional *ex situ* examination of crack paths derived using synchrotron x-ray computed tomography, to determine the microstructural features that underlie the toughness of bone and how this can degrade with biological factors.

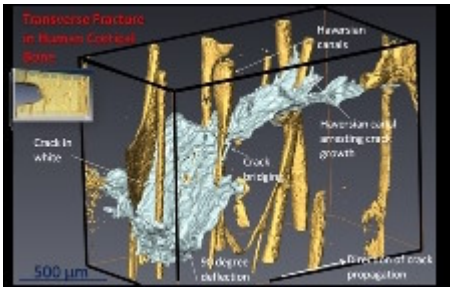

Figure : Three-dimensional synchrotron x-ray computed tomograph of a crack path in human cortical bone (humerus, 37 yr donor), in the transverse orientation, showing toughening induced by crack deflection and twisting (Barth and Ritchie).

046

NANOSCALE DEFORMATION MECHANISMS IN BONE

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Bone is an organic collageneous matrix reinforced with thin plate-like apatite crystallites at the nanoscale [1] as well as a small fraction of noncollagenous matrix proteins and proteoglycans. Such an organic – inorganic composite has both high stiffness as well as excellent fracture resistance, but these properties can be significantly reduced, with adverse clinical consequences, during bone diseases and ageing. To clarify the role of the nanostructure in bone fracture resistance we applied micromechanical techniques that probe the real time deformation of the mineralized fibrils and mineral nanocrystallites under tensile load, using high brilliance synchrotron radiation. Our results show that the strain is passed down the hierarchy in successively lower fractions – a ratio of 12:5:2 from the tissue to the fibril to the mineral particle level [2]. Such a cooperative and hierarchical deformation, which is sensitive to the degree of hydration of the organic matrix, shields the brittle mineral phase from the largest applied stresses and maintains bone integrity. As a result the 3 – 5 nm thick mineral nanoparticles are very strong, bearing more than 3 times the failure load of bulk hydroxyapatite before breaking. In the inelastic regime, thermal activation analysis is used to show that the basic failure event is localized to within about 0.6 nm^3 and with an activation enthalpy of ~ 1.1 eV [3]. The magnitude of these quantities suggests that breaking of ionic bonds (possibly calcium mediated) between extrafibrillar polyelectrolyte molecules may be the rate limiting elementary step in bone plasticity. At the microscale, using digital image correlation to measure strains, we show that the transition from elastic to inelastic deformation corresponded with the appearance of several large-strain deformation bands across the specimen [4].

(1) P. Fratzl et al, J. Mater. Chem. 14 (2004) 2115
(2) H. S. Gupta et al, Proc. Natl. Acad. Sci. 103 (2006) 17741
(3) H. S. Gupta et al, J. Roy. Soc. Interface 4 (2007) 277
(4) G. Benecke et al, J. Mater. Res., in press (2009)

047

TGF-BETA AND BONE MATRIX MATERIAL PROPERTIES

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The material properties of extracellular matrices contribute to a tissue's mechanical integrity and are a source of cues that direct cellular behavior. Given their importance to the biological and structural function of a tissue, these material properties are tissue-specific and carefully defined. Even within the skeleton, bone matrix material properties are anatomically distinct, such that the bone matrix of the cochlea is harder than the bone matrix of the femur or calvarial bone. However, the mechanisms that establish a tissue's distinctive material properties remain unclear.

Transforming growth factor beta (TGF-β) has emerged as a growth factor that can regulate the elastic modulus of bone, skin, and other extracellular matrices during development. Inhibition of TGF-β signaling, through the TGF-β receptor complex and its downstream effector, Smad3, increased bone matrix elastic modulus, as well as several other bone properties. TGF-β inhibits osteoblast differentiation by Smad3-dependent repression of Runx2. In the same manner, TGF-β targets the function of Runx2 to define bone matrix elastic modulus and hardness as assessed by nanoindentation. Downstream transcriptional targets of TGF-β and Runx2 that are responsible for the differences in these material properties remain under investigation.

Whether changes in bone matrix material properties could be achieved only by developmental alteration of TGF-β function remained unclear. To examine the role of TGF-β in the postnatal skeleton, we evaluated the effects of pharmacological inhibition of the TGF-β type I receptor (TβRI) kinase on bone mass, architecture and material

properties. Inhibition of TβRI function had both anabolic and anti-catabolic effects that increased bone mass, improved trabecular architecture, and increased bone matrix mineral concentration and material properties. The coordinate regulation of multiple bone parameters by inhibition of TGF-β function also resulted in increased bone fracture resistance, suggesting the therapeutic potential for targeting the TGF-β pathway to treat conditions of skeletal fragility.

048

STRATEGIES FOR PREVENTING FALLS IN THE ELDERLY.

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The prevention of falls and fall related fracture is an ongoing challenge for many countries across the world. Over the last 14 years there have been approximately 120 RCTs looking at whether it is possible to prevent falls and fall related injury in older people. A reasonably robust evidence base now exists to support a number of approaches to falls prevention including exercise, OT home assessment, cataract extraction, medication modification and a multifaceted approach to prevention. The evidence also suggests that approaches need to be population specific and therefore knowledge of the literature is required to ensure individuals are offered an intervention for which there is an anticipated benefit.

Prevention of fall related fracture in older people is a key priority area for many health care services given the associated costs both to the individual and to the health care system. Addressing bone health in isolation of falls risk factors is unlikely to represent the most cost-effective approach to fracture prevention and there is an imperative for those interested in fracture prevention to develop models of care that address both the bone related and falls related factors for fracture.

049

BONE MICROARCHITECTURE AND FRACTURE RISK

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Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass and microstructural deterioration of bone tissue with a consequent increase in bone fragility. The importance of trabecular and cortical bone microstructure to bone strength is well documented. Therefore evaluation of both microarchitecture and BMD may improve estimation of the risk of fracture. However, until recently the only way to assess bone microarchitecture was via histomorphometric analysis of iliac crest biopsies. Recently, noninvasive imaging methods, including high-resolution peripheral quantitative computed tomography (hr-pQCT) and high-resolution magnetic resonance imaging (hr-MRI), that allow in vivo 3D assessment of bone microstructure at peripheral skeletal sites have been developed. In addition, multi-detector QCT and flat-panel CT are being used to assess trabecular and cortical bone architecture.

Recent studies indicate that in postmenopausal women, vertebral and non-vertebral fractures are associated with low volumetric bone density, architectural alterations of trabecular and cortical bone. These alterations are partially independent of aBMD assessed by DXA, suggesting that assessment of bone microarchitecture in vivo may provide improved sensitivity and/or specificity in identifying individuals at greatest risk for fracture.

With a goal of outlining the potential clinical utility of these imaging modalities, this lecture will review the clinical studies with focus on three areas — use of bone architecture measurements to: 1) gain new insight to the patterns of age- and disease-related bone deterioration; 2) identify individuals at greatest risk for fracture; and 3) monitor treatment efficacy. The use of micro-finite element analysis based on hr-pQCT images will also be reviewed. The current data provide strong rationale for prospective studies to determine the utility of assessing microarchitecture for predicting the risk of osteoporotic fractures, as well as monitoring disease progression and the response to treatments.

050

VITAMIN D, FALLS AND FRACTURES – WHAT'S NEW?

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Vitamin D deficiency and insufficiency are strongly associated with osteoporosis and fracture. Vitamin D also seems important to muscle function, cell proliferation and differentiation, and deficiency is probably related to falls risk.

Vitamin D is a steroid hormone precursor produced photochemically in skin and the dietary requirement of Australian lifeguards is zero. Conversely in housebound older people all requirements must be met from diet. The active hormone, calcitriol, results from two hydroxylation steps, the second regulated by parathyroid hormone (PTH). Additionally, there are two compounds known as "vitamin D" – cholecalciferol (D3) found in animals and ergocalciferol (D2) produced by fungi. These differ significantly in potency. One of the problems besetting the quite heated discourse on vitamin D has been confusion over which molecule(s) is/are being studied.

Many studies show that vitamin D insufficiency is highly prevalent in older people with falls and fractures. However, treatment studies have had mixed results with some showing large benefit, others showing no effect and a few suggesting harm. Secondary research has been no more helpful with systematic reviews reaching opposite conclusions, sometimes even when appraising the same primary studies.

In a word, the problem is heterogeneity – of subjects and interventions.

There is a world of difference in falls and fracture risk between healthy Third Agers and frail nursing home residents. Falls risk itself is heterogeneous. A keen older gardener with arthritic knees may have the same absolute risk as a frail care-home resident with dementia, but the risk factors are completely different.

Similarly, the effect of vitamin D – an inactive precursor – is quite unlike that of calcitriol, a powerful calciotropic hormone. Study interventions accordingly range from the inadequate to the excessive.

Further analysis taking these factors into account is ongoing, but at present supplementation is best targeted at the highest-risk groups rather than populations.

051

CONTRIBUTION OF GENETIC FACTORS TO THE INDIVIDUALIZED PROGNOSIS OF FRACTURE

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One of the major priorities in osteoporosis research is to develop valid models for identifying individuals at high-risk of fracture for allocating appropriate medical intervention. In the last few years it has been realised that a single arbitrary cut-off point value of bone mineral density is unlikely to describe the true risk of fracture, and that a multiparametric approach may be more useful for identifying high-risk individuals. Because each individual is a unique case, the prognosis of fracture risk should be individualized. One way to define the uniqueness or individuality of prognosis is to combine genotypes and clinical risk factors.

Several genetic variants have recently been shown to be associated with fracture risk, however, the effect size was modest with relative risk varying between 1.1 and 2.0. With such a modest association, any single genetic variant may not be clinically useful since it is not sufficient to improve risk prediction in an individual. Genetic profiling with multiple genetic variants can help improve the individualized prognosis of fracture. A profile of up to 100 genetic variants is required to accurately predict the risk of fracture (area under the operating characteristic curve of 0.80) for an individual. However, the population attributable fraction is low because few individuals are exposed to multiple high-risk genotypes. Nevertheless, in a population with lifetime risk of fracture being 50%, and if a genetic profile increases the risk of fracture by 2-fold, then the number needed for genetic effect (NNG) is only 2. Thus, when the genetic effect is expressed in terms of NNG, it is clear that a genetic profiling can have a clinically meaningful effect. Moreover, since fracture tends to segregate within families, a genetic profiling in high-risk families can yield a higher effectiveness than in the general population.

Prognosis is about imparting absolute risk to an individual. Because every individual is unique, the ultimate risk assessment and therapeutic strategy should be based on the genetic and non-genetic profiles that are relevant to the individual. Our analysis suggests that genetic variants can enhance the prognosis of fracture and improves the efficiency and effectiveness of a case-finding strategy.

052

FUTURE MANAGEMENT OF OSTEOPOROSIS

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This presentation will focus on pharmacologic therapy. Major limitations of current treatments include poor compliance, and limited efficacy of antiresorptive therapy for nonvertebral fractures, especially for long-term therapy.

Ideally, patients who are initially noncompliant will be treated with periodic parenteral therapy. The combination of potent bone-forming agents followed by antiresorptives may 'cure' osteoporosis. The availability of potent bone forming therapy will raise a challenging issue: how much bone mass is enough?

Bone densitometry and risk factors are widely used to find people who warrant drug therapy. Patients with vertebral fractures warrant treatment, but most are not diagnosed or treated. Systematic screening of older patients for vertebral fractures could identify a large number of patients who would substantially benefit from treatment.

053

A FUSION PROTEIN OF PARATHYROID HORMONE (PTH) AND A COLLAGEN BINDING DOMAIN SHOWS SUPERIOR EFFICACY AND LONGER DURATION OF ACTION COMPARED TO PTH(1-34) AS AN ANABOLIC BONE AGENT IN NORMAL FEMALE MICE.

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Despite application of current therapies, osteoporosis remains a worldwide problem. Osteoporotic hip fractures cost ~18 billion dollars per year in the US alone. Anabolic agents such as parathyroid hormone show superior efficacy to antiresorptives (i.e. bisphononates), but have not been widely accepted in part because of inconvenient dosing (daily injection). We have developed a fusion protein of parathyroid hormone PTH(1-33) and a collagen binding domain derived from Clostridium histolyticum (CBD) (PTH-CBD), designed to be retained in bone and extend the duration of the anabolic bone effect. In vitro studies showed PTH-CBD binds collagen and activates the PTH/PTHrP receptor similar to PTH(1-34). We compared the efficacy and duration of PTH-CBD to PTH(1-34) and vehicle control in normal young female C57BL/6J mice (Jackson Laboratories). Using molar equivalent doses of PTH-CBD and PTH(1-34), monthly intraperitoneal dosing of PTH-CBD (320 mcg/kg) for 6 months achieved a higher peak BMD than did 2 weeks of daily administration of PTH(1-34) (80 mcg/kg) (PTH-CBD: 81±3, PTH(1-34): 75±3, vehicle: 64±2 mg/cm2, $p<0.05$). These increases in BMD were sustained for an additional 6 months following the dosing interval (PTH-CBD: 71±3, PTH(1-34): 63±3, vehicle: 54±2 mg/cm2, $p<0.05$). Serum alkaline phosphatase levels were elevated in the PTH-CBD group at the end of the study, showing continued drug effect 7 months after the last dose and indicating an anabolic mechanism of action (PTH-CBD: 118±49, PTH(1-34): 40±18, vehicle: 32±8 IU/L, $p<0.05$). In a separate study, a single dose of PTH-CBD (320 mcg/kg) showed superior efficacy to 2 weeks of daily administration of PTH(1-34) (80 mcg/kg) (PTH-CBD: 76±2 vs. PTH(1-34): 71±2, vehicle: 67±1 mg/cm2, $p<0.05$). The gains in BMD were sustained for 12 months following the injection of PTH-CBD, while the BMD in the group which received PTH(1-34) returned to control levels (PTH-CBD:79±2, PTH: 66±1, vehicle: 63±1 mg/cm2, $p<0.05$). There was no additional benefit observed with administration of a PTH-CBD every three months vs. a single injection. The overall results suggest that a single dose of PTH-CBD provides superior efficacy and longer duration of action compared to daily PTH(1-34) in increasing BMD in normal female mice through an anabolic mechanism.

054

SKELETAL ANABOLIC ACTIVITY OF CANNABINOID RECEPTOR AGONISTS

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An increasing number of studies demonstrate the occurrence of a skeletal cannabinoid system. This system consists of CB1 cannabinoid receptors expressed in skeletal sympathetic nerve terminals and CB2 receptors in osteoblasts and

osteoclasts. In addition, bone contains relatively high levels of endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) and the endocannabinoid metabolic enzymes N-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD), diacylglycerol lipase (DAGL) and fatty acid amide hydrolase (FAAH). Ablation of either CB leads to a low bone mass phenotype. We show here that sympathetic signaling stimulates osteoblastic DAGL expression and 2-AG synthesis. In turn, 2-AG activates CB1 that restrains norepinephrine (NE) release from sympathetic terminals, thus alleviating the adrenergic inhibition of bone formation. Hence, skeletal NE and 2-AG levels are regulated by a negative feedback circuit. The downstream signaling cascade of osteoblastic CB2 consists of Gi-protein and Erk1/2 activation that lead to transcriptional upregulation of Mapkapk2 followed by CREB activation. In addition, activated CB2 is associated with decreased inhibitory-SMAD expression, and increased SMAD1,5,8 phosphoryltion. The CB2 agonist, HU-308, rescued bone loss in a mouse ovariectomy model at 20 mg/Kg/day for 6 weeks. Combined µCT/histomorphometric analysis in these mice indicated that the increase in trabecular bone volume density (BV/TV) in the lumbar vertebrae and distal femoral metaphyses is mainly by stimulating bone formation and augmenting trabecular thickness. To assess the effect of cannabinoids on fracture healing, adult male rats were treated daily with a mixture of tetrahydrocannabinol (THC) and cannabidiol, the most abundant constituents of marijuana and hashish. Administration of these cannabinoids, each at 5 mg/Kg/day, commenced immediately after standardized femoral pinning and mid-diaphyseal fracturing. µCT assessment carried out after 2 weeks showed 37% increase in the BV/TV of the newly formed mineralized callus, accompanied by 32% increase in trabecular number and, particularly important, 52% stimulation of the trabecular connectivity density. Together, these results portray cannabinoid receptor agonists as potent bone anabolic agents. Further studies characterizing the skeletal activity of selective CB2 agonists are suggested for the rescue of skeletal deficits, since this receptor is not involved in the well established cannabinoid psychotropic effects and control of energy metabolism.

055

EFFECTS OF DENOSUMAB AND ALENDRONATE ON SKELETAL MICROARCHITECTURE

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The rate of bone remodeling is a major determinant of bone loss, structural decay, and fracture risk. Denosumab, a fully human monoclonal anti-RANKL antibody, reversibly inhibits remodeling, increases BMD, and reduces fracture risk (1). DXA has shown that denosumab increases BMD more than alendronate throughout the skeleton (2). This study explored the trabecular, cortical, and microstructural effects of these treatments in more detail.

In a double-blind, placebo-controlled phase 2 pilot study, 247 ambulatory postmenopausal women (mean age 61 yrs) with mean lumbar spine T-score of -2.4 were randomized to denosumab (60mg sc 6-monthly, n=83), alendronate (70 mg weekly, n=82), or placebo (n=82). All received daily calcium (≥500mg) and vitamin D (≥400 IU). Changes in morphology at the radius and tibia were assessed using high-resolution peripheral quantitative computed tomography (HR-pQCT) . Markers of turnover and safety were also evaluated.

After 12 months, total, trabecular, and cortical BMD and cortical thickness decreased in the placebo arm. These decreases were prevented with denosumab and blunted with alendronate (Table). The treatment effect was larger with denosumab for some of these variables. Similar trends occurred at the distal tibia (not shown). Denosumab resulted in rapid (≤1 week) reduction of turnover markers. The frequency and types of adverse events were similar in each group (94% placebo 92% denosumab, 95% alendronate).

Denosumab prevented cortical thinning, as well as decreases in trabecular density and cortical density (a surrogate of intracortical porosity) as measured by HR-pQCT at the distal radius and tibia. These data advance our understanding of the structural consequences of bone loss and highlight potential differences in microarchitectural outcomes of these treatments.

Table. Least Squares Mean (95% CI) Percent Change From Baseline at 12 Months at the Distal Radius by ANCOVA

	Placebo	Denosumab (60mg 6-monthly)	Alendronate (70mg weekly)
Total BMD	-2.1 (-2.7, -1.4)	1.1 (0.5, 1.8)	0.0 (-0.6, 0.7)
Trabecular BMD	-2.0 (-2.9, -1.0)	0.5 (-0.5, 1.5)	-0.6 (-1.6, 0.4)
Cortical BMD	-1.5 (-1.8, -1.2)	0.3 (-0.1, 0.6)	-0.3 (-0.6, 0.0)
Cortical thickness	-0.8 (-1.8, 0.3)	3.4 (2.2, 4.5)	2.4 (1.2, 3.5)

(1) Cummings et al. ASBMR 2008; oral presentation #1286
(2) Brown et al. JBMR. 2008;doi10.1359/jbmr.080910

056

A DOUBLE-BLIND, PLACEBO-CONTROLLED, PHASE III STUDY OF BAZEDOXIFENE/CONJUGATED ESTROGENS IN POSTMENOPAUSAL WOMEN: EFFECTS ON BONE MINERAL DENSITY

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Background: Bazedoxifene/conjugated estrogens (CE) is a novel tissue selective estrogen complex (TSEC) partnering bazedoxifene, a selective estrogen receptor modulator (SERM), and the CE found in Premarin®. The goal of the TSEC is to attain the best of both estrogen therapy (ET) and SERMs for menopausal symptom treatment and the prevention of postmenopausal osteoporosis.

Objective: To evaluate the effect of bazedoxifene/CE on bone mineral density (BMD) in postmenopausal women.

Methods: This randomized, double-blind, placebo-controlled, multicenter, phase III trial evaluated 6 doses of bazedoxifene/CE administered daily for 2 years (bazedoxifene 10, 20, or 40 mg, each with CE 0.45 mg or CE 0.625 mg). Placebo and raloxifene 60 mg served as controls. The primary objective was to evaluate the incidence of endometrial hyperplasia; secondary objectives included evaluation of BMD at the anteroposterior lumbar spine and hip as assessed by dual energy x-ray absorptiometry (DXA).

Results: The trial evaluated 3,397 postmenopausal women of which 2,315 were enrolled in 2 osteoporosis substudies: 1,454 women more than 5 years since menopause and 861 women at least 1 year but less than or equal to 5 years since menopause. At baseline, no significant differences were found between the treatment groups in mean age, race, body mass index, or years since last menstrual period. At 2 years, all strengths of the TSECs were associated with greater increases in BMD at both spine and hip compared with placebo ($P \leq 0.001$). In both substudies, TSECs containing 20 mg bazedoxifene were associated with greater increases in lumbar spine BMD than raloxifene ($P < 0.05$) at 2 years, and all TSECs were associated with greater changes in serum bone markers (C-telopeptide, osteocalcin) at 2 years compared with placebo (all $P < 0.001$).

Conclusions: While SERMs have demonstrated positive effects on the skeleton, they have not attained the magnitude of BMD changes observed with ET or bisphosphonates. Partnering of appropriate doses of bazedoxifene with CE was associated with greater increases in BMD of lumbar spine and hip at 2 years compared with placebo or raloxifene in postmenopausal women, coupled with efficacy on menopausal symptoms not achieved by SERMs alone.

057

EFFICACY OF RISEDRONATE AGAINST HIP FRACTURE IN PATIENTS WITH NEUROLOGICAL DISEASES: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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OBJECTIVE: Neurological diseases including Alzheimer's disease, stroke, and Parkinson's disease have been reported to increase the risk for fractures. The purpose of the present study was to clarify the efficacy of risedronate against hip fracture in patients with neurological diseases. METHODS: The literature was searched with PubMed from 1995 to the present, with respect to strictly conducted randomized controlled trials (RCTs) with narrow confidence intervals (CIs),

and a meta-analysis was conducted. RESULTS: Four RCTs met the criteria; one RCT for Alzheimer's disease (n=461, mean age: 78 years), two RCTs for stroke (n=267, mean age: 76 years for men; n=345, mean age: 71 years for women), and one RCT for Parkinson's disease (n=223, mean age: 71 years). According to the results of RCTs, the relative risks (95% CI) of hip fracture with risedronate treatment compared with placebo treatment were 0.26 (0.10, 0.69) for Alzheimer's disease, 0.20 (0.04, 0.89) for stroke in men, 0.14 (0.02, 1.16) for stroke in women, and 0.34 (0.09, 1.21) for Parkinson's disease. Overall, the relative risk (95% CI) for hip fracture with risedronate treatment was 0.25 (0.13, 0.48), suggesting 75% of risk reduction rate with risedronate treatment in patients with one of the three neurological diseases (heterogeneity: 0.58, P=0.9016 and overall effect: 17.36, P<0.0001). No severe adverse events were reported in the risedronate and placebo groups. CONCLUSION: The results of a meta-analysis of strictly conducted RCTs suggest that there is efficacy against hip fracture and safety with risedronate treatment in patients with neurological diseases including Alzheimer's disease, stroke, and Parkinson's disease.

058

IMPACT OF ALFACALCIDOL ON BONE MINERAL DENSITY (PQCT&DXA) OF RADIUS, TIBIA, SPINE AND HIP IN POSTMENOPAUSAL ALENDRONATE TREATED WOMEN WITH REDUCED BONE MASS

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Objectives: Assessment of the impact of alfacalcidol (alfa) on cortical and trabecular bone mineral density (BMD) and bone strength (Strength Strain Index = SSI), measured with peripheral quantitative computed tomography (pQCT), and bone mass of spine and hip, measured with dual energy X-ray absorptiometry (DXA) in postmenopausal women treated with alendronate 70mg once weekly (ALN) + 500mg calcium daily.

Subjects and Methods: 279/282 postmenopausal women (ITT population) in the age of 73.6 ± 4.7 years with low bone mass (mean T-score -2,4SD at baseline) treated with ALN + calcium have been recruited. In a randomized, double-blinded, placebo controlled, bi-centric study these patients received either 1µg alfacalcidol or placebo (PLC) per day, additionally. BMD was measured at forearm and tibia with pQCT (XCT2000 Stratec, Pforzheim) as well as with DXA (QDR Delphi, Hologic) at spine and hip over a period of 36 months.

Results: Cortical BMD in mid-shaft tibia (pQCT) increased in the alfa/ALN group significantly compared to PLC/ALN group. The relative increase of cortical BMD in the PLC/ALN group was 53.1% less than that of the alfa/ALN group ($p = 0.043$). Similar results were in all other cortical skeletal areas as well. The alfa/ALN group showed also a significant gain in distal tibia trabecular BMD ($p = 0.002$). In the PLC/ALN group trabecular bone loss was detected. DXA-BMD of the spine (L1-4) increased in both groups (6.99% alfa/ALN vs. 4.64% PLC/ALN). The gain in spine was 33.3% lower in the PLC/ALN group as compared to the alfa/ALN group. The SSI (at tibia 38% region) increased significantly in the alfa/ALN group as opposed to the PLC/ALN group, in which the effect was by 78.6% lower than that in the alfa/ALN group.

Conclusion: Alfacalcidol significantly meliorates the efficacy of alendronate treatment in relation to DXA-BMD of spine, cortical BMD of radius and tibia, as well as trabecular BMD and SSI of the tibia. The increase of cortical BMD in these patients treated with alfacalcidol 1µg daily and alendronate + calcium has a desirable effect on bone strength and, consequently, on fracture risk.

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EFFECTIVE OSTEOPOROSIS TREATMENTS REDUCE MORTALITY: A META-ANALYSIS

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Introduction: Fragility fractures cause significant morbidity and mortality. Effective osteoporosis treatment can significantly reduce the incidence of fractures, but it is not known whether such treatment reduces mortality.

Objective: We set out to determine whether effective osteoporosis treatment reduces mortality.

Data sources: We searched Medline and the Cochrane Central Register of Trials prior to September 2008, and 2000-2008 ASBMR conference abstracts for eligible trials.

Review methods: Eligible studies were randomized placebo-controlled trials of approved doses of medications with proven efficacy in the prevention of both vertebral and non-vertebral fractures, in which the study duration was >12 months and there were >10 deaths. Trials of estrogen and selective estrogen receptor modulators were specifically excluded.

Results: 8 eligible studies of 4 agents (risedronate, strontium ranelate, zoledronic acid, and denosumab) were included in the primary analysis. In 2 alendronate studies, the treatment dose changed during the study, and thus these studies were only included in secondary analyses. In the primary analysis, treatment was associated with an 11% reduction in mortality [relative risk (RR) 0.89, 95% confidence interval (CI) 0.80-0.99, P=0.036]. In the secondary analysis of all 10 studies, the results were similar (RR 0.90, 0.81-1.0, P=0.044). Mortality reduction was not related to age or incidence of hip or non-vertebral fracture, but was greatest in trials conducted in populations with higher mortality rates.

Conclusions: Effective treatment for osteoporosis reduces mortality by approximately 10%. This reduction in mortality provides further rationale for treating frail elderly patients at risk of fracture.

060

LIFESTYLE IMPACT ON LIFETIME BONE LOSS IN WOMEN AND MEN. THE TROMSØ STUDY

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Background and aim of study: A physically active, non-smoking lifestyle including a body mass index (BMI) > 18 kg/m² may reduce bone loss in adults and elderly women and men. The effect of different lifestyles on lifetime bone loss has not yet been estimated. With data from a large population based survey in Norway, the aim of this study was to estimate the impact of different lifestyles on peak bone mass, lifetime changes in bone mineral density (BMD) and fracture risk in men and women.

Method: BMD (g/cm²) was measured at distal and ultradistal forearm sites with single X-ray absorptiometric (SXA) - devices in 3390 men and 4558 women aged 24-84 years in 1994-95. After 6.5 (SD 0.3) years, in 2001, measurements were repeated in 2557 men and 3625 women. Lifestyle information was collected through questionnaires. Non smoking, physically active and BMI = 25 kg/m^2 was defined as "healthy" and smoking > 20 a day, inactivity and BMI < 18 kg/m^2 as "unhealthy" lifestyles. The statistical analyses included linear mixed models, usage of fractional polynomials expanded with the lifestyle factors, in addition to estimations of differences in fracture risk between lifestyle groups.

Results : At peak in the forth decade there were no statistically significant differences in BMD levels between lifestyle groups, but at the age of 80, healthy men and women had lost respectively 15 and 35 percent BMD, at the distal site. In comparison, unhealthy men and women lost more than 25 and 46 percent, respectively, at the same site. With the corresponding lifestyles, healthy men and women lost respectively 16 and 36 percent BMD at the ultradistal site, and those with an unhealthy lifestyle, lost 27 and 56 percent, respectively. Differences in bone loss from peak age until 80 years corresponds to an estimated 85% increased risk of forearm fracture in women with an unhealthy compared to a healthy lifestyle, at the distal site. The corresponding figure in men is 69%. The corresponding estimated risks for all types of fracture is 48% in women and 39% in men with an unhealthy compared to a healthy lifestyle.

Conclusion : A lifestyle which includes non smoking, high physical activity level and high body weight preserves bone mass and reduces fracture risk in both sexes from peak bone mass to old age.

061

DO MEN AND WOMEN HAVE SIMILAR ABSOLUTE RISK OF FRACTURE?

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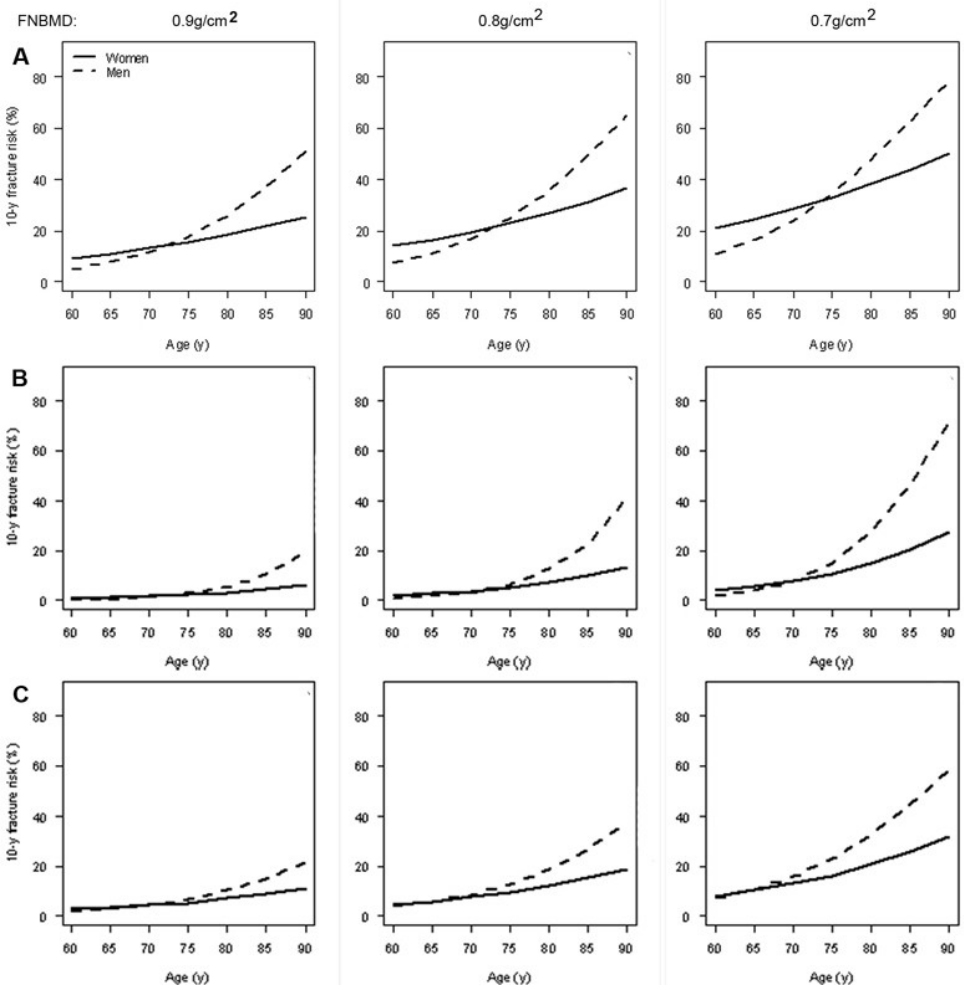
It has been assumed that for given age and bone mineral density (BMD), women men have similar absolute risk of fracture. This study sought to examine that assumption by estimating the absolute risk of fracture for men and women.

Data from 2216 participants (1358 women) aged 60+ years as at 1989 were analyzed. Femoral neck BMD was measured by DXA (GE-Lunar) at baseline. The incidence of fragility fractures was ascertained between 1989 and 2007. Five-year and 10-year risks of fracture were then calculated for each age and BMD level separated by sex using the Cox's proportional hazards model.

For a given age, femoral neck BMD in men was greater than in women (0.98g/cm^2 vs 0.85 g/cm^2 and 0.88g/cm^2 vs. 0.75 g/cm^2 at 60 and 75 years of age, respectively) . During the follow-up period, 426 women (31.4%) sustained a fragility fracture, and significantly higher than in men (149 fractures, incidence 17.4%). However, there is an interaction between age and sex, such that among those aged less than 75 years, for any given BMD level, men had a lower risk of fracture than women; on the other hand, among those aged 75 or above, men tended to have higher risk of fracture (Figure).

Thus, these data suggest that the assumption used in the derivation of T-scores for the diagnosis of osteoporosis men is untenable, because for a given BMD T-score, the risk of fragility fracture in men is not the same as in women. These data reinforce the need for the prognosis of fracture to be individualized and differently for men and women.

Figure: 10-y risk of any fracture (A), hip fracture (B) and clinical vertebral fracture (C) in women and men grouping according to average (0.9g/cm^2), low (0.8g/cm^2) and very low (0.7g/cm^2) femoral neck BMD values.



062

FRACTURE RISK AND ACCESS TO FUNDED OSTEOPOROSIS TREATMENTS IN NEW ZEALAND, AUSTRALIA, UK AND CANADA

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Aim: The aim was to map absolute 10-year risks of major osteoporotic (MFR) and of hip (HFR) fractures to access criteria for funded osteoporosis treatment in New Zealand and to compare these with Australia, UK and Canada.

Method: Clinical risk indicators (age, gender, height, weight, smoking, alcohol use, fragility fracture, parental hip fracture, rheumatoid arthritis, glucocorticoid use) were recorded prospectively from patients attending for DEXA scanning. Anonymised data from 272 untreated patients with both an evaluable DEXA scan and a completed questionnaire from a convenience sample of 400 was used. Absolute 10 year risks of major osteoporotic (MFR) and hip (HFR) fractures were estimated using the WHO FRAX calculator (UK database). The eligibility of each patient for access to osteoporosis treatment was categorized using funding criteria for New Zealand (PHARMAC), Australia (PBS), UK (NICE TA 2008) and Canada. The medians and 95% confidence intervals for those eligible Vs ineligible were calculated. Thresholds for eligibility were estimated to lie midway between the upper bound of the 95% CI of those ineligible and the lower bound of the 95% CI of those eligible.

Results:

	NZ	NZ	Aus	Aus	UK	UK	Canada*
%	MFR	HFR	MFR	HFR	MFR	HFR	MFR
Eligible n	100	100	98	98	29	29	131
Median	15.0	4.1	15.0	3.2	23.0	11.0	15.0
95% CI	13.4-16.6	3.1-5.1	13.4-16.6	2.1-4.3	19.9-26.3	8.8-13.2	13.9-16.1
inelig. n	172	172	174	174	243	243	141
Median	8.1	0.9	8.1	1.0	8.9	1.3	6.8
95% CI	7.4-8.9	0.5-1.3	7.2-8.9	0.6-1.4	8.2-9.6	1.0-3.0	6.5-7.1
Threshold	11.2	0.9	11.2	1.0	14.8	5.9	10.5*

*In Canada, access to treatment varies between states; "considered" where MFR > 10%

Conclusion: Despite differences in the criteria between Australia and New Zealand, patients gaining access to treatment have nearly identical risk profiles and eligibility thresholds. Canadian criteria define a population group at slightly lower risk but allow many more patients to access treatment. UK recommendations deny access to at least two thirds of patients eligible for treatment in Australia, New Zealand or Canada and define a patient group with a higher risk profile.

063

CORTICAL BONE: NEGLECTING SAMSON'S HAIR AND ACHILLES' HEEL.

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Trabecular bone loss contributes to bone fragility. However, cortical bone accounts for 50% of vertebral strength and determines a long bone's resistance to bending. About 80% of bone is cortical, 80% of fractures are non-vertebral and occur at predominantly cortical sites. Cortical bone is not 'compact'; its many osteons, their cement line, concentric lamellae of differing collagen orientations and mineral densities form structurally heterogeneous barriers against crack initiation and propagation: Sampson's hair.

Bone loss erodes these structures. The amount lost is determined by (i) the remodeling rate, (ii) the imbalance between the bone volumes resorbed and formed by each remodeling event, (iii) the bone volume available to be lost and (iv) its accessibility to being remodeled. The fourth factor is determined by the surface area of the three (endocortical, trabecular and intracortical) components of its inner (endosteal) envelope because remodeling requires a surface to be initiated upon (figure).

Trabecular bone's large surface area exposes it to high remodeling so it is lost rapidly. Cortical bone's low surface/volume ratio makes it less accessible to being remodeled so it is lost slowly. As age advances, remodeling on trabecular surfaces removes them with their surfaces while remodeling on the endocortical and intracortical (haversian canals) surfaces increases them by endocortical tunneling and coalescence of intracortical resorption cavities forming large pores, particularly adjacent to the marrow; Achilles' heel. Total bone remodeling does not change or increases but mainly occurs upon cortical surfaces so now the large cortical volume (80% of bone) is accessible to being

remodeled and so is lost; 70% of the bone lost during aging is cortical and most is lost from the intracortical not endocortical surface. Intracortical porosity destroys bone's hierarchical organization leaving a thin porous cortex less resistant to crack initiation and propagation. Trivial age-related periosteal apposition is no defense against this decay. Cortical bone loss is neglected and accounts for most bone loss; its surfaces are a target for drug therapy.

064

RANK LIGAND INHIBITION: EXPERIENCES FROM RODENTS TO PRIMATES.

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Bone remodelling involves the RANK/RANK Ligand (RANKL)/osteoprotegerin (OPG) pathway, with osteoclasts requiring RANKL for their differentiation, activation and survival. OPG works as a native inhibitor of RANKL, binding it, therefore preventing binding to its receptor RANK and blocking bone resorption. One hypothesis of the pathophysiology of osteoporosis suggests that elevated levels of RANKL relative to OPG may result in bone loss. Rectifying this imbalance by increasing OPG levels relative to RANKL or at least increasing the inhibition of RANKL, is a potential therapeutic pathway. Denosumab, an investigational fully human monoclonal antibody to human RANKL, mimics the effects of OPG on bone resorption but with the advantage of a longer half-life.

Preclinical testing of denosumab required the development of huRANKL knockin mice that express chimeric human/murine RANKL that recognize denosumab as denosumab is not effective against murine RANKL. Denosumab had no effect on bone resorption or bone mass in young wildtype mice, whereas denosumab reduced bone resorption and increased cortical and trabecular bone mass in young huRANKL mice. Denosumab also reduced bone turnover and increased bone volume and trabecular thickness in aged female huRANKL mice. In OVX huRANKL mice, denosumab increased BMD and improved cancellous bone structure to a greater extent when compared to alendronate.

To address the question of whether the observed increases in bone mineral density (BMD) are associated with increases in bone strength, a study was designed to determine the long-term effects of denosumab on bone turnover, BMD, and bone strength in aged, ovariectomized cynomolgus monkeys.[1] Treatment with denosumab in this animal model resulted in reduced bone turnover and increases in bone mass, volumetric BMD and bone strength at cortical and cancellous sites. No significant changes were found in bone material properties.

These animal models confirm the effectiveness of RANKL inhibition using denosumab and are valuable for further characterizing denosumab activity.

1. Ominsky MS et al. JBMR 2007; Vol. 22 (Suppl. 1): S23

065

RANK LIGAND INHIBITION IN PATIENTS WITH POSTMENOPAUSAL BONE LOSS

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Patients with osteoporosis have an increased risk of fracture, and the goal of treatment is to reduce this risk. One measure of osteoporosis severity has been bone mineral density (BMD) with lower BMDs being associated with greater risk of fracture. WHO defines osteoporosis as a BMD value of T-score $\leq$ -2.5 standard deviations below the mean value of a young healthy population. While initially acceptable as a clinical endpoint, fracture risk reduction has become a key measure of efficacy for regulatory agencies. Further, fracture risk reduction at all sites, including hip, vertebral and other sites (e.g. humerus, tibia, fibula, distal forearm), are important because of the burden associated with these fractures. Therefore, in assessing clinical efficacy, reduction of fracture risk at all skeletal sites represents an important treatment goal.

RANK ligand is an essential mediator of osteoclast formation, function and survival throughout the skeleton. Denosumab, an investigational, fully human monoclonal antibody, targets RANK ligand, thereby inhibiting osteoclasts. Early clinical trials of denosumab in postmenopausal women with bone loss demonstrated decreased bone resorption and increased BMD at vertebral, hip and distal forearm sites. Late stage clinical trials of denosumab examined fracture risk reduction. One of these trials was presented recently[1]. This trial known as FREEDOM (Fracture REduction Evaluation of Denosumab in Osteoporosis every 6 Months) study is a randomised, double-blind, placebo-controlled multicenter phase 3 study involving 7,868 postmenopausal osteoporotic women aged 60-90 years old from 214 clinical centres in 32 countries. These women had to have a lumbar spine or total hip T-score $<$ -2.5 and $\geq$ -4.0. Patients received either receive 60 mg s.c. denosumab, or matching placebo, every 6 months for 3 years as well

as daily calcium (at least 1000 mg/day) and vitamin D (at least 400 IU/day). The primary endpoint was the risk of new vertebral fractures and secondary outcomes included the risk of non-vertebral fractures and hip fractures.

1. Cummings et al. Presented at ASBMR 2008, Sept. 16 2008, Montreal, A 1286

066

WNT AND HH SIGNALING IN SKELETAL DEVELOPMENT AND HOMEOSTASIS

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Wnt and Hedgehog signaling are major regulators of vertebrate skeletal development and homeostasis. Using genetic approaches in the mouse, we have demonstrated that the Wnt/β-catenin signaling controls the differentiation of progenitor cells into either osteoblasts or chondrocytes. Genetic ablation of β-catenin in the developing mouse embryos resulted in ectopic formation of chondrocytes at the expense of osteoblast differentiation during both intramembranous and endochondral ossification. In contrast, ectopic upregulation of the canonical Wnt signaling led to suppression of chondrocyte formation and enhanced ossification. Like Wnt signaling, several other signaling pathways also play critical roles in controlling skeletal development. To gain a full picture of the molecular regulatory network of skeletal development, it is essential to understand how the Wnt/β-catenin signaling is integrated with the other signaling pathways in controlling various aspects of skeletal development. We have found that Wnt and Ihh signaling interact with each other in distinct ways to control osteoblast differentiation, chondrocyte proliferation, hypertrophy and survival in the developing endochondral bone. We also found that the Hedgehog signaling controls adult bone homeostasis by regulating bone turn over through PTHrP and RANKL.

067

NEUROGENIC REGULATION OF OSTEOBLAST FUNCTION

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Osteoporosis is caused by a failure of bone homeostasis. The precise molecular mechanism controlling bone homeostasis is largely unknown. Increasing evidences that neurons and neurotransmitters are intimately involved in bone remodeling shed light on a novel regulatory mechanism for bone homeostasis. Namely, like all other homeostatic functions, bone remodelling is under the control of hypothalamus.

I will summarize the current understanding of genetic, molecular and physiological bases for the central control of bone remodeling and discusses the future directions of this new research field.

068

HYPOXIA-INDUCIBLE FACTOR 2A (HIF-2A) CONTROLS SEQUENTIAL STEPS OF ENDOCHONDRAL OSSIFICATION DURING SKELETAL GROWTH AND OSTEOARTHRITIS PROGRESSION

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Purpose: The late stage of endochondral ossification including chondrocyte hypertrophy, cartilage matrix degradation, and vascular invasion are known to be crucial not only in physiological skeletal growth, but also in cartilage destruction and osteophyte formation during osteoarthritis progression. Since the mechanism underlying these coordinated sequential steps remains an enigma, this study sought to identify the transcription factor and the related signals that control the stage.

Methods&Results: A screening of transcription factors using ATDC5 cells and HeLa cells transfected with a luciferase-reporter construct containing a promoter of type X collagen (COL10) revealed hypoxia-inducible factor 2

alpha (HIF-2 α) as the strongest transactivator. Real-time RT-PCR showed that HIF-2α expression increased during differentiation of ATDC5 cells in association not only with COL10 expression, but also with expressions of matrix metalloproteinase 13 (MMP13) and vascular endothelial growth factor (VEGF), crucial factors for matrix degradation and vascular invasion, respectively. Immunohistochemistry showed that HIF-2α was localized in the hypertrophic zones of the growth plate, and that the expression of HIF-2α was strongly detected in the degenerated articular cartilage but not detected in the normal articular cartilage. Heterozygous HIF-2α-deficient (HIF-2α+/-) mice exhibit dwarfism with impairment of the late stage of endochondral ossification and decreased expressions of COL10, MMP13 and VEGF. Moreover, cartilage destruction was markedly inhibited in the experimental osteoarthritis model of HIF-2α+/- mice. Functional studies using stable lines of ATDC5 cells with retroviral introduction revealed that expressions of COL10, MMP13 and VEGF, as well as ALP and Alizarin red stainings were enhanced by the HIF-2α overexpression, and that these markers were suppressed by overexpression of the HIF-2α dominant negative mutant or the small interfering RNA. Luciferase assay , electrophoretic mobility shift assay and chromatin immunoprecipitation assay confirmed the respective resposive elements of HIF-2α in COL10, MMP13 and VEGF genes.

Conclusions: HIF-2α is the crucial transcription factor that controls the late stage of endochondral ossification and plays essential roles in the progression of osteoarthritis through direct transactivation of COL10, MMP13 and VEGF, suggesting this factor being a therapeutic target of growth retardation and osteoarthritis.

069

IDENTIFICATION OF A TRANSCRIPTION FACTOR P63 FOR JOINT CARTILAGE FORMATION BY ANALYSES OF WNT9A AND GDF5 PROMOTERS

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To understand the molecular mechanism underlying joint cartilage formation is an essential step for a regenerative medicine against skeletal disorders like osteoarthritis. Wnt9a and Gdf5 are known to be representative molecules for joint formation from mouse genetics findings. Using comparative mapping between human and mouse Wnt9a promoters within 3 kb upstream of the transcriptional initiation site, we detected a highly conserved region between -158 bp and -117 bp, called Joint-Specific Enhancer (JSE). Screening from a phage display library of human tracheal cartilage by biopanning with tandem copies of the JSE construct identified a p53 family gene p63 as the most probable transcription factor for this region. In fact, the JSE contained a p53 family-binding motif. In both chondrogenic ATDC5 cells and non-chondrogenic HuH-7 cells transfected with luciferase-reporter constructs containing tandem copies of the JSE, the p63 overexpression stimulated the transcriptional activity depending on its repeat number, which was abrogated by site-directed mutagenesis in the binding motif above. Electrophoretic mobility shift assay (EMSA) showed binding of nuclear extracts from p63-overexpressed HuH-7 cells with the JSE oligonucleotide probe. The complex disappeared with an excess of unlabelled probe and underwent supershift by the p63 antibody, indicating a specific binding between JSE and p63. Overexpression of p63 increased the endogenous Wnt9a expression in ATDC5 and HuH-7 cells by real-time RT-PCR. Similarly, deletion, mutagenesis, and tandem-repeat analyses of the luciferase assay within the 3 kb Gdf5 promoter identified a core responsive element to p63 between the -1 bp to +63 bp region containing the binding motif. EMSA also showed the specific binding between this region and p63. Furthermore, the p63 overexpression in de-differentiated mouse costal cells caused chondrogenic differentiation with induction of endogenous type II collagen (COL2), SOX6 and SOX9 expression. In vivo expression of p63 was detected in all cartilaginous tissues of developmental limbs of mouse embryos by immunohistochemistry . When we further investigated the skeletons of p63-deficient (p63-/-) mouse embryos, they exhibited severe short limb deformities with suppressed COL2, SOX6 and SOX9 expressions. Taken together, the present analyses of Wnt9a and Gdf5 promoters identified p63 as a crucial transcription factor for joint cartilage formation. Further understanding of the molecular network around p63 will be a great benefit for achievement of cartilage regenerative medicine.

070

LOSS OF A SINGLE *BIM* ALLELE RECOVERS THE DEFECTIVE OSTEOCLAST FUNCTION IN *BCL-2-/-* MICE BUT DOES NOT RESTORE THE ANABOLIC ACTION OF PTH

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Anti-apoptotic molecule Bcl-2 resides on the mitochondrial outer membrane, and inhibits apoptosis by suppressing cytochrome *c* release from mitochondria. We previously reported that Bcl-2 promotes the differentiation, activation and survival of both osteoblasts and osteoclasts by analyzing *bcl-2-/-* mice. However since *bcl-2-/-* mice die about 3 weeks of age due to renal failure and immunodeficiency, the *in vivo* analysis of the role of *bcl-2* deficiency in the skeletal tissue of adult mice is still hampered. We therefore generated *bcl-2-/-bim+/-* mice in which a single *bim* allele was knocked out and compared them with their *bcl-2+/-bim+/-* littermates. Both mice grew normally into adults without causing the polycystic kidney disease or growth retardation. The bone mineral density was significantly reduced in *bcl-2-/-bim+/-* mice compared to *bcl-2+/-bim+/-* mice, while *bcl-2-/-bim+/-* mice exhibited normal bone resorption as determined by bone histomorphometric analysis. Osteoclasts generated from *bcl-2-/-bim+/-* bone marrow cells displayed normal bone-resorbing activity, while the mineralization activity of *bcl-2-/-bim+/-* osteoblasts was impaired. This indicates that the inactivation of a single *bim* allele restores the defective function caused by *bcl-2*-deficiency in osteoclasts, but is not enough to rescue the defect in *bcl-2*-deficient osteoblasts. We also found that single dose PTH stimulation increased Bcl-2 expression in wild type osteoblasts *in vitro*. We then examined the effect of intermittent PTH treatment on bone mass in the mice. While PTH treatment markedly increased the trabecular bone mass in *bcl-2+/-bim+/-* mice, it did not affect the bone mass in *bcl-2-/-bim+/-* mice. Intermittent PTH treatment did not affect the bone formation rate in *bcl-2-/-bim+/-* mice although *bcl-2-/-bim+/-* mice remarkably increased the osteoid surface. Furthermore, PTH did not increase the serum level of osteocalcin in *bcl-2-/-bim+/-* mice whereas no significant difference in serum level of CTx was observed between *bcl-2+/-bim+/-* and *bcl-2-/-bim+/-* mice. These data indicate that the mineralization of the bone matrix produced by *bcl-2-/-bim+/-* osteoblasts is attenuated after intermittent PTH treatment. Collectively, we demonstrated that Bcl-2 promotes differentiation, activation and survival of both osteoblasts and osteoclasts, and that Bcl-2/Bim balance is critical for osteoclast function. In addition, anabolic activity of PTH requires Bcl-2 expression in osteoblasts.

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MICRO RNA 18A REGULATES CHONDROCYTIC PHENOTYPE: INVOLVEMENT OF *CCN2/CTGF* AS A MAJOR TARGET GENE

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Micro RNA (miRNA) is a major class of non-coding RNAs that are involved in a variety of biological events including development of a number of tissues in higher eukaryotes. In order to identify miRNAs that regulate endochondral ossification, we searched for miRNA candidates that were down-regulated in chondrocytic cells and related to CCN family protein 2/connective tissue growth factor (CCN2/CTGF). CCN2 is a classic member in CCN family proteins. CCN family proteins consist 6 members; CCN1 (Cyr 61), CCN2 (CTGF), CCN3 (NOV), CCN4 (WISP1), CCN5 (WISP2), and CCN6 (WISP3). These proteins are composed of 4 distinct modules, i.e., IGF-binding protein-like module (IGF-BP), von Willebrand factor type C repeat (VWC), thrombospondin type-1 repeat (TSP1), and C-terminal module (CT). With these modules, CCN proteins interact with a number of intra- and extracellular molecules, such as proteoglycans, growth factors including transformin growth factor (TGF)- b , and adhesion proteins and modulate diverse cellular functions including chemotaxis, differentiation, and apoptosis as a signal conductor. CCN2 has been known to promote endochondral ossification and cartilage regeneration. We selected miRNA candidates targeting *Ccn2* by a combination of microarray and *in silico* analyses. Five miRNAs were predicited to target the *Ccn2* 3'-UTR. Among those candidates, expression of miR-18a was found to be the most strongly repressed in chondrocytic cells. Utilizing reporter gene constructs and a synthetic mature miR-18a duplex, we experimentally confirmed a miR-18a target in the same region in the 3'-untranslated region (UTR) of human *Ccn2* as predicted *in silico*. Also, the introduction of the miR-18a duplex efficiently repressed the production of CCN2 in those cells. Interestingly, this *Ccn2* silencing was conferred entirely at a translation stage without affecting the steady-state mRNA level in chondrocytic

HCS-2/8 cells; whereas accelerated degradation of *Ccn2* mRNA has been observed in human breast cancer MDA-231 cells. Finally, transfected miR-18a duplex significantly caused the repression of the mature chondrocytic phenotype. Our present study revealed a regulatory role for miR-18a in chondrocytic differentiation through CCN2 and a variable mode of post-transcriptional regulation of the same miRNA, which was dependent on the cellular background.

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A TRANSCRIPTION FACTOR ZNF219 REGULATES CHONDROGENESIS BY FORMING A TRANSCRIPTION FACTORY COMPLEX WITH SOX9.

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Sox9, a HMG-containing transcription factor, is essential for chondrogenesis. Mutations of the SOX9 in human are associated with Campomelic dysplasia characterized by disturbed skeletal development. Sox9 directly modulates the expression of chondrogenic genes including type II collagen (COL2a1), type XI collagen (COL11a2) and aggrecan in collaboration with Sox5 and Sox6. However, the mechanism by which Sox9 regulates chondrogenesis is not fully understood, especially, the component that cooperates with Sox9 through forming transcription factory has not been determined yet. In this study, we attempted to identify a transcriptional partner of Sox9 and examined its functional roles in chondrogenesis . We constructed a full-length cDNA library from the mouse chondrogenic cell line, ATDC5, and screened the cDNA library using a luciferase reporter construct driven by COL2a1 promoter that is hooked with Sox9 binding element. We consequently isolated the Znf219 gene based on its novelty and primary structure. Whole mount *in situ* hybridization demonstrated specific spatial expression of Znf219 in the developing limb buds in which COL2a1 and Sox9 were also strongly co-expressed. Znf219 mRNA was also expressed in primary chondrocytes. Znf219 stimulated COL2a1 gene promoter activity through direct binding to the COL2a1 promoter. Znf219 markedly enhanced the transcriptional activity of Sox9 for the COL2a1 gene promoter. Co-immunoprecipitation experiments showed Znf219 physically associated with Sox9. DsRed-tagged Znf219 well co-localized with Venus-tagged Sox9 in the nuclei. These results collectively suggest Znf219 forms a transcription factory with Sox9 in the regulation of chondrocyte differentiation. Overexpression of Znf219 in C3H10T1/2 mesenchymal cells using the adenovirus system profoundly increased Sox9-induced mRNA expression of COL2a1, aggrecan and COL11a2. Conversely, knockdown of Znf219 using micro RNA system decreased Sox9-induced mRNA expression of these molecules. Furthermore, introduction of a dominant-negative mutant Znf219 inhibited BMP2-induced chondrogenesis in C3H10T1/2 cells and mouse embryo limb bud cells . In conclusion, we isolated a novel transcription factor Znf219 that plays an important role in the regulation of chondrocyte differentiation as a molecular partner of Sox9 through forming transcription factory. Further characterization of Znf219 would deepen our understanding of the regulatory mechanism of chondrogenesis.

073

C/EBPB / P57^{KIP2} SIGNALING MAINTAINS TRANSITION FROM PROLIFERATION TO HYPERTROPHIC DIFFERENTIATION OF CHONDROCYTES DURING SKELETAL GROWTH

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This study investigated the role of CCAAT/enhancer-binding protein β (C/EBPβ), a multi-functional transcription factor, in chondrocytes during skeletal growth. C/EBPβ was shown by immunohistochemistry to be localized mainly in late proliferative and pre-hypertrophic chondrocytes of the mouse growth plate. The homozygous deficient (C/EBPβ−/−) mice exhibited dwarfism from embryonic stages. The growth plate showed elongation of the proliferative zone and delay of the chondrocyte hypertrophy determined by BrdU uptake and type X collagen (COL10) immunostaining, respectively. In cultures of primary chondrocytes from C/EBPβ−/− ribs, cell proliferation determined by XTT assay was enhanced, while hypertrophic differentiation determined by COL10, MMP-13 and VEGF mRNA

levels as well as ALP and Alizarin red stainings was suppressed. In contrast, retroviral overexpression of C/EBPβ in the wild-type chondrocytes suppressed proliferation and enhanced hypertrophic differentiation, suggesting the involvement of C/EBPβ in the transition from proliferation to hypertrophic differentiation of chondrocytes. A DNA cell cycle histogram in C3H10T1/2 cells revealed that C/EBPβ overexpression caused accumulation of cells in G0/G1 fraction. A microarray analysis identified several cell cycle factors as transcriptional targets of C/EBPβ, and among them $p57^{Kip2}$, a cyclin-dependent kinase inhibitor, was shown by real-time RT-PCR to be most strongly up-regulated by the C/EBPβ overexpression and down-regulated by the C/EBPβ deficiency (C/EBPβ−/−) in chondrocytes. p57 was co-localized with C/EBPβ in the growth plate, which was dramatically decreased by the C/EBPβ deficiency. In HuH-7 cells transfected with a luciferase-reporter gene construct containing the p57 promoter region, the transcriptional activity was enhanced by C/EBPβ co-transfection. Deletion, mutagenesis, and tandem-repeat analyses of the luciferase assay identified the core responsive element to be between the −150 and −130 bp region containing a putative consensus site for C/EBP. The electrophoretic mobility shift assay revealed the binding of nuclear extracts of C/EBPβ-overexpressed COS7 cells with the oligonucleotide including this region, whose specificity was verified by the C/EBPβ antibody supershift. Finally, knockdown of p57 by siRNA inhibited the C/EBPβ-induced hypertrophic differentiation in cultured chondrocytes. We conclude that C/EBPβ directly transactivates p57 at a C/EBP element to maintain transition from proliferation to hypertrophic differentiation of chondrocytes during skeletal growth.

074

ANDROGEN/ANDROGEN RECEPTOR DIRECTLY ACTS IN TRABECULAR OSTEOCLASTS AND CORTICAL OSTEOBLASTS

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Sex steroid hormones regulate bone homeostasis through their specific nuclear receptors, Estrogen Receptors (ERs) and Androgen Receptor (AR). Our previous report suggested that estrogen prevents trabecular bone loss through osteoclastic ERα with facilitating Fas ligand expression and osteoclast apoptosis, however this investigation was only revealed in female osteoclastic ERα knockout mice (Nakamura and Imai et al. Cell 2007). On the other hand, male conventional AR knockout mice (ARKO) exhibited bone loss in both trabecular and cortical bone (Kawano and Sato et al. PNAS 2003). However, ARKO male had female type internal and external genital grand and exhibited endocrine disorders such as low testosterone and high luteinizing hormone (LH). From these reasons, it has been unclear whether bone loss of ARKO caused by AR disfunction in bone tissue directly or indirectly. To clarify direct functions of AR in bone tissue, we generated both osteoclast- and osteoblast-specific conditional ARKO mice (OcARKO and ObARKO) using Cathepsin K-Cre mice and Col1a1(2.3kb)-Cre mice mating with AR floxed mice. Body weight of OcARKO and ObARKO was not different from control mice, regardless of gender. There is no statistical difference between both conditional ARKO mice and control mice in endocrine parameters, such as testosterone, estradiol, follicle stimulating hormone (FSH) and LH. From the results of analyses for bone phenotypes, both OcARKO and ObARKO female mice exhibited no apparent differences when compared to the control female mice. Whereas in male mice, at 8 weeks of age, OcARKO exhibited osteopenia in trabecular bone such as distal femur and a high rate of turnover in bone metabolism. However, cortical bone mineral density was not altered in OcARKO male. On the other hand, DXA analysis revealed that ObARKO male exhibited decreased bone mineral density (BMD) in diaphysis of long bones and calvaria, which are especially osteoblasts rich portion in bone tissue. Furthermore, femoral cortical thickness of ObARKO was significantly decreased by μCT analysis, compared to the control. Although more examinations should be needed, our results partly suggested that androgen could directly regulate bone cells, especially in trabecular osteoclasts and cortical osteoblasts.

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NKX3.2 IS AN IMPORTANT MEDIATOR OF HYPOXIA-INDUCED CHONDROCYTIC DIFFERENTIATION

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Recently, we reported that hypoxia promotes chondrocytic differentiation in mesenchymal lineage independently of Sox9 (J Biol. Chem. 281(41), 2006). Other papers also described that there is Sox9- independent pathway in hypoxia-induced chondrocytic differentiation(J Cell Biol. 177(3), 2007) (J Biol. Chem. 283(8), 2007). It is known that Nkx3.2, a novel chondrogenic transcription factor induced by Shh, suppresses Runx2 in mesenchymal lineage. Then, we hypothesized that hypoxia induces Nkx3.2 activation followed by Runx2 suppression, leading to promotion of chondrocytic differentiation, while osteoblastic differentiation is inhibited. C3H10T1/2 cell was cultured under normoxia (20% O_2) and hypoxia (5% O_2) with rh-BMP2 (300ng/ml). At first, we performed immunocytochemistry for Nkx3.2 protein using micromass culture samples. Nkx3.2 was expressed at the site of cell condensation and the number of nucleus with positive staining was increased by hypoxia. Real time RT-PCR analysis revealed that *Nkx3.2* gene expression was promoted by hypoxia from day1 to 10. On the other hand, *Runx2* expression was suppressed from day3 to 7. *Shh* gene expression was up-regulated from 6hrs to 24hrs after hypoxic stimulation. *Sox9* gene expression showed no difference between normoxia and hypoxia. Furthermore, because it is known that PTH-rP also positively regulates Nkx3.2 during endochondral ossification, we checked the gene expression of *PTH-rP* and found that it was promoted by hypoxia, while *Ihh*, a prehypertrophic chondrocyte marker, was down-regulated. From these observations, it is suggested that hypoxia up-regulates Shh and Nkx3.2 and induces subsequent Runx2 down-regulation, which in turn promotes chondrocytic commitment and inhibits chondrocyte hypertrophy during endochondral ossification. Next, we examined the relationships between Nkx3.2 and Runx2 in hypoxia. Over expression of Nkx3.2 using wild-type Nkx3.2(WT-Nkx3.2) down-regulated Runx2 gene expression and Runx2 transcriptional activity was also suppressed in luciferase reporter assay. On the other hand, we also checked the specificity of Nkx3.2 in hypoxia using RNAi. Alcian blue staining showed that siRNA for Nkx3.2 abolished hypoxia-induced glycosaminoglycan (GAG) production of 10T1/2. These results suggest that Nkx3.2 is a dominant regulator of chondrocytic differentiation induced by hypoxia, and that Nkx3.2-induced Runx2 down-regulation is the primary mechanism of suppression of osteoblastic differentiation. Although further confirmation on the relationship between Nkx3.2 and Runx2 in hypoxia is necessary, Nkx3.2 seems to play important roles in hypoxia-induced Runx2 down-regulation, inhibition of osteoblastic commitment and promotion of chondrocytic differentiation.

(1) J Biol. Chem. 281(41), 2006
(2) J Cell Biol. 177(3), 2007
(3) J Biol. Chem. 283(8), 2007

076

FUNCTIONAL MORPHOLOGY OF OSTEOCYTES

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Recently, osteocyte death was reported after a single injection of diphtheria toxin (DT) in transgenic mice that express the DT receptor specifically on osteocytes. Following osteocytes' death, histological assessment revealed not only trabecular bone loss but also peripheral demineralization of osteocytic lacunae in cortical bone. Those findings suggest that osteocytes may be involved in the regulation of bone remodeling and maintenance of appropriate levels of bone minerals. In a physiological state, the regular arrangement of the osteocytic lacunar-canalicular system (OLCS) appears to be related to the system's functionality. Accordingly, histochemical examinations demonstrated plump ALPase-positive osteoblasts and many TRAPase-positive osteoclasts on the metaphyseal primary trabeculae, featuring an irregularly arranged OLCS. On the other hand, secondary metaphyseal trabeculae displayed regular OLCS arrangement. The endosteal region of cortical bones close to the metaphysis showed faster bone deposition and irregular OLCS, with diaphyseal regions displaying slower bone deposition and regularly arranged OLCS. It appears that bone remodeling, especially the speed of bone deposition, affects the regularity of OLCS. Immunolocalization of FGF 23 and DMP-1 were investigated and compared with the OLCS regularity. DMP-1 was evenly localized in all OLCS regardless of its regularity. FGF23 expression, however, was more intensely localized in osteocytes from cortical bones with regularly arranged OLCS than in metaphyseal trabeculae. Within the cortical bone, the periosteal region, which displays an irregular OLCS, revealed a less amount of FGF23. Alternatively, the endosteal region exhibited intense FGF23 immunoreaction along with its regular OLCS. Thus, a regularly arranged OLCS appears to be

a totally functioning system that synthesizes FGF23. In this presentation, we will share our recent findings on osteocyte morphology.

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MOLECULAR BIOLOGY OF THE OSTEOCYTE

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As the osteocyte progenitor cell on the bone surface differentiates into an early osteocyte, a unique process occurs, that of mineralization. During this time the cell has undergone a dramatic change in shape and gene expression. Genes/proteins thought to be important in the change from a cuboidal to a dendritic morphology include E11/gp38, cdc42, destrin, CapG, and others. Genes important in the mineralization process include Pex and Dmp1 as deletion or mutation in either of these genes results in hypophosphatemic rickets due to an elevation of FGF23 in osteocytes.

Whereas E11/gp38 is a marker for the early osteocyte, *Sost*/sclerostin is a marker for the late or mature osteocyte surrounded by a mineralized matrix. This gene/protein has served to bring the osteocyte into general interest due to its exclusive location in mature osteocytes in the adult skeleton and the clinical development of bone anabolic agents that neutralize the function of this protein. Sclerostin is a direct inhibitor of Lrp5, a key receptor/signaling molecule in the Wnt/ b -catenin pathway. Another inhibitor of this pathway expressed in osteocytes includes Dkk1 and as no specific Wnt activators have been identified, it has been proposed that these inhibitors provide a “brake” to a constitutive anabolic Wnt pathway in osteocytes.

Hormonal regulation as through PTH and mechanosensation/transduction provides a means to release this “brake” to bone formation by down regulating sclerostin and Dkk1 expression. Early responses to mechanical loading include calcium signaling within seconds, followed by nitric oxide, ATP production, and prostaglandin release soon thereafter. All four of these small molecules are essential for an anabolic response of bone to loading. Prostaglandin signals through multiple pathways including the Wnt pathway. Signaling through the prostaglandin/wnt pathway appears to be responsible for gap junction function, for the maintenance of osteocyte viability and resistance to apoptotic factors, and potentially for the down regulation of wnt pathway inhibitors in response to mechanical loading.

Whereas key molecular pathways have been identified in osteocytes regarding phosphate homeostasis and mechanical loading, little is known how osteocytes recruit osteoclasts or regulate calcium metabolism. RANKL and M-CSF are most likely involved in osteocyte recruitment and activation of osteoclasts, but at this time, little is known regarding how osteocytes can remove and replace their perilacunar matrix during lactation or in response to hormones. The molecular biology of the osteocyte has become a new frontier for bone research.

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OSTEOCYTE CONTROLS PI HOMEOSTASIS

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The osteocyte is a star-shaped bone cell that differs from the cuboidal morphology of its progenitor cell (osteoblast) in numerous ways. Osteocytes are networked to each other, to osteoblasts and to osteoclasts via long dendrites (cytoplasmic extensions). This cell is generally known and accepted as the “professional” mechanosensory cell, sending signals of (re)modeling in response to loading. Yet, our knowledge of osteocyte biology is very limited in comparison with what we know about osteoblasts and osteoclasts. This gap in our knowledge is due to the following: 1) Osteocytes are encased in the mineralized extracellular matrix, which makes studying them extremely difficult; 2) Osteocytes are not capable of mitotic division and have a life-span over a decade with much low synthetic activity; 3) Very few genes are osteocyte-specific, which limits our research resources; 4) There is only one osteocyte cell line (MLO-Y4, generated by Lynda Bonewald), and it does not express important genes that the primary osteocytes have, such as DMP1; and 5) There is a lack of interest in osteocyte biology. In this talk, I will 1) show osteocyte morphology using a variety of microscopes [scanning electron microscope (SEM), backscattered SEM, transmission electron microscope, and confocal microscope]; 2) provide evidence that osteocytes are actively involved in mineralization using a mouse model with and without a gene named *Dmp1* that is highly expressed in the osteocyte; 3) provide strong evidence demonstrating a close connection between blood vessels and osteocytes; and 4) use animal models to show that osteocytes are endocrine cells that control phosphate homeostasis (key talk). This concept goes beyond the current

dogma that the kidneys control the phosphate homeostasis through the parathyroid hormone and 1,25 (OH)2 vitamin D3 and that bone is viewed as the key targeting organ due to its “phosphate reservoir”.

079

EPIDEMIOLOGY OF VERTEBRAL FRACTURES

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Vertebral fractures (VF) are the most common osteoporotic fractures but investigations into their etiology and epidemiology are difficult because their definition remains controversial, the majority of VF do not come to clinical attention and few develop acutely. The prevalence of VF increases with age in both men and women, no matter the definition but there are substantial differences in the actual frequency depending on the definition. The lifetime risk of a clinically diagnosed VF is 16% in white women and 5% in white men. In contrast to hip fractures, the worldwide variability of VF is considerably lower. VF, even radiographic VF, have important consequences including back pain, disability and death. Prevalent VF are the strongest risk factor for future fractures including hip, new vertebral and other fractures even over a 15 year period. Low BMD in particular lumbar spine is a strong predictor of future VF. Clinical risk factors for VF include older age, previous fracture, low body mass index, smoking, low calcium intake and physical activity. To test the hypothesis that the WHO 10-yr probabilities of hip and other osteoporotic fracture (FRAX) predict incident radiographic VF over the short (3.5 years; n=5680) and long (15 years; n=2121) term, we used data from the Study of Osteoporotic Fractures (SOF). Incident VF were identified from lateral spine radiographs and defined as a decrease of at least 20% and 4mm at any vertebral level. The 10-yr probabilities of hip and other osteoporotic fractures were calculated by the WHO group. The WHO models were significant predictors of incident radiographic VF: odds ratio (95% confidence intervals) of VF (short term) per one standard deviation increase in the probability of other osteoporotic fracture with BMD was 1.84 (1.66, 2.04) or without BMD, 1.65 (1.49, 1.83); for VF over the long term, with BMD, 1.68 (1.51, 1.87) and without BMD, 1.52 (1.37, 1.68). Associations were weaker for the hip fracture probability models. Stratification by prevalent VF and by age revealed similar results. These results suggest that the FRAX model may be useful in identifying women at risk for VF.

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NEW APPROACHES TO THE DIAGNOSIS OF VERTEBRAL FRACTURES

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Vertebral fractures (VF) are the most common fractures associated with osteoporosis. They are strong predictors of risk of future fracture (X5 for vertebral fracture; X2 for hip fracture). Bone protective & enhancing therapies reduce the risk of future VF by 40%-70% for quite modest concomitant increases in bone mineral density (BMD). So identification of VF is important to the appropriate management of patients with osteoporosis. There is evidence that VF are under-diagnosed, which is partly related to a high proportion (30%-50% or more) being asymptomatic, but also to VF not being reported accurately & clearly on radiographs & other imaging techniques. This stimulated the joint Vertebral Fracture Initiative of International Osteoporosis Foundation (IOF) & European Society of Skeletal Radiology (ESSR) in 2002. The aim was to raise the profile of osteoporosis generally, & vertebral fractures in particular, amongst radiologists, & an educational CD was created & distributed, being available at http://www.iofbonehealth.org .

Spinal radiographs are still the most common imaging method for the diagnosis of VF; images need to be performed in a standardized way to avoid artefacts which may mimic VF (‘bean can effect' due to tilting of vertebrae). The definition and quantification of VF varies; some methods are based simply on change in vertebral shape (e.g. 6 point morphometry) designated as ‘deformity'; others are based on both shape & appearance (semi-quantitative method of Genant et al; Algorithm-based qualitative [ABQ] method of Jiang et al). These various methods & definitions account for differences in VF prevalence in epidemiological & clinical studies. Normal variants (‘cupid's bow') & pathologies (trauma, Scheuermann's disease, Schmorl's nodes, infection, degenerative disease) other than VF can change vertebral shape & must be differentiated from VF.

VF may be identified as incidental findings on other radiographic procedures, lateral images obtained on dual energy X-ray absorptiometry DXA scanners (Instant Vertebral Assessment IVA) & other imaging modalities (computed tomography CT, magnetic resonance imaging MRI, radionuclide scans RNS); the latter can differentiate if VF are old

or new (relevant to vertebroplasty), or due to more sinister pathology (myeloma, metastases). Radiographic & IVA images have the potential for automatic computer diagnosis of VF.

081

VERTEBROPLASTY AND KYPHOPLASTY

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Spinal augmentation, or the percutaneous infusion of medical cement, has been widely applied for treatment of painful, osteoporotic fractures. The two most commonly applied types of augmentation are vertebroplasty and balloon-assisted vertebroplasty, or Kyphoplasty. Until recently, clinical data was primarily limited to case series and nonrandomized trials. These data showed nearly universal, outstanding treatment efficacy. Pain severity was routinely diminished after the procedure, and reduced pain levels persisted for up to two years. Scores on validated measures of back-pain related disability also improved routinely in these series.

Notwithstanding these many positive reports, important questions have remained unanswered. These questions relate not only to the efficacy of the procedure but also to long term safety. Regarding efficacy, the available data have failed to clarify the exact mechanism of action of the cement in achieving pain relief, with leading and still unproven mechanisms including stabilization of microfractures and heat-mediated denervation. The case series and existing nonrandomized trials have failed to demonstrate the impact of factors, other than the cement, that might affect outcome. These factors include patient and physician expectation of pain relief, regression toward the mean, the natural history of healing fractures, and the impact of local anesthesia and patient positioning on the procedure table. Regarding safety, the long term impact of indwelling cement on subsequent vertebral fracture has remained unclear.

Several recently-completed, randomized, prospective spinal augmentation trials have offered important new data about the procedure. The Fracture Reduction Evaluation (FREE) Trial randomized, in non blinded fashion, 300 patients between medical therapy and Kyphoplasty, with 2 year follow up[1]. Two randomized, blinded, placebo controlled trials of vertebroplasty have recently been completed, including The Efficacy and Safety of Vertebroplasty for Treatment of Painful, Osteoporotic Vertebral Fractures [2] as well as the INvestigational Vertebroplasty Efficacy and Safety Trial (INVEST) [3] . These trials offer new insight into not only the true efficacy of the cement, from the placebo-controlled trials, but also into the long term safety of the procedure, from the FREE study.

Relevant results from these trials will be discussed to enhance understanding of the safety and efficacy of spinal augmentation, and to offer insight into future research directions for the field.

(1) The Fracture Reduction Evaluation (FREE) Trial. Clinicaltrials.gov unique identifier: NCT00211211

(2) Buchbinder R, et al. Efficacy and safety of vertebroplasty for treatment of painful osteoporotic vertebral fractures

(3) Gray LA, et al. INvestigational Vertebroplasty Efficacy and Safety Trial (INVEST)

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MANAGEMENT OF OSTEOGENESIS IMPERFECTA

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Children with moderate to severe forms of osteogenesis imperfecta (OI) require adequate physiotherapy, rehabilitation and orthopedic surgery. Supportive treatment with bisphosphonates can improve the effects of these nonmedical treatment modalities. Benefits of bisphosphonate treatment include decreased pain, lower fracture incidence, and better mobility. Among the various bisphosphonates, intravenous pamidronate has been studied in most detail. Newer intravenous bisphosphonates, such as neridronate and zoledronate, are easier to administer and will be increasingly used in the future. The role of oral bisphosphonates in the treatment of OI is unclear at present, as the largest placebo-controlled study on this topic found little clinical benefit. The optimal treatment regimen and the long-term consequences of bisphosphonate treatment in children are currently unknown. Given these uncertainties, treatment with bisphosphonates during growth should be reserved for patients who have significant clinical problems, such as vertebral compression fractures or long-bone deformities. Medical therapies other than bisphosphonates play a minor role at present. Gene-based therapy currently remains in the realm of preclinical research.

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CCN2/CTGF HAS ANTI-AGING EFFECTS TO PROTECT ARTICULAR CARTILAGE FROM AGE-RELATED DEGENERATIVE CHANGES.

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Objective: CCN family 2 / Connective tissue growth factor (CCN2/CTGF) promotes proliferation and differentiation of chondrocytes and enhances production of extracellular matrices during the endochondral ossification. To examine the role of CCN2/CTGF in maintenance of the cartilage phenotype, we generated transgenic mice which overexpress CCN2/CTGF under the *Col2a1* promoter and analyzed articular cartilage from aged mice.

Methods: To overexpress CCN2/CTGF in cartilage, HA-tagged *ccn2/ctgf* cDNA and *IRES-LacZ* as an expression marker were cloned under the 6kb *Col2a1* promoter-enhancer. Expression of transgene in articular cartilage was detected by X-gal staining and overexpression of *ccn2/ctgf* mRNA was monitored by real-time PCR analysis using RNAs prepared from articular cartilage of new born and 2 weeks-old mice. Knee joints from littermates of 21-months old (5 transgenic mice (TG); male 1, female 4, and 1 wild type (WT); male) and 18-months old (WT; male 3, female 2) were analyzed radiographically and histologically. For histological analysis, safranin-O fast green staining and immunostaining using anti-type I, II, X collagens, and MMP13 antibodies were carried out.

Results: X-gal staining of articular cartilage showed expression of transgene in new born and 2 weeks-old TG. Overexpression of *ccn2/ctgf* mRNA was also detected in new born and 2 weeks-old TG. To examine the effects of overexpression of CCN2/CTGF in articular cartilage, radiographic analysis of knee joints from littermates of 21-months and 18-months old mice was performed. As a result, 50% (3 out of 6) of WT, but not TG, had narrowing joint space and rough cartilage surfaces diagnosed as osteoarthritic changes. Histological analysis of articular cartilage from WT showed less safranin-O staining than transgenic cartilage. Furthermore, immunohistochemical analysis showed enhanced staining for type X, I collagen, and MMP13 in WT cartilage as compared to transgenic cartilage.

Conclusion: Cartilage-specific overexpression of CCN2/CTGF during developing and growth period reduced osteoarthritic changes in aged articular cartilage. Thus CCN2/CTGF may play a role as an anti-aging factor by stabilizing articular cartilage.

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INHIBITORY ROLE OF GAQ / PKCΔ SIGNAL IN THE BONE ANABOLIC ACTION OF PTH

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Contrary to the well-known bone anabolic action of the Gαs signal in osteoblasts, we have reported that the Gαq signal inhibits bone formation by analyses of transgenic mice with osteoblast-specific overexpression of a constitutively active Gαq transgene (CA-Gαq). The present study sought to examine the involvement of the Gαq signal in the bone anabolic action of PTH. Although subcutaneous intermittent injection of rhPTH (1-34) caused about a 10% increase of bone volume in wild-type mice, this effect was not seen in the CA-Gαq transgenic littermates. In the culture of osteoblastic MC3T3-E1 cells, stable overexpression of the CA-Gαq inhibited their differentiation determined by ALP, which was abrogated by addition of GF109203X, an inhibitor of protein kinase C (PKC). Among 13 PKC family members, PKCδ was shown to be most strongly induced by the CA-Gαq overexpression by immunoblotting. We further created double knockout (DKO) mice deficient in osteoblast-specific Gαq and global Gα11 by crossing COL1-Cre transgenic mice and mice with Gαq gene flanked with loxP / global ablation of Gα11 (Gαq-fl/fl;Gα11−/−). Although histomorphometric analyses revealed that bone volume and turnover of the DKO mice were normal under physiological conditions, the bone anabolic effect of rhPTH injection was significantly enhanced in the DKO mice compared with that in the wild-type littermates, confirming inhibition of the PTH action by endogenous Gαq signaling. In the culture of primary mouse calvarial osteoblasts, intermittent treatment of rhPTH stimulated differentiation

determined by ALP and mRNA levels of osteogenesis markers such as COL1A1 and osteocalcin. These effects were markedly enhanced in the cells from the Gαq/Gα11 DKO mice, which were restored by addition of PMA, an activator of PKC, to the levels of wild-type osteoblasts. Subcellular translocation of PKCδ to membrane enhanced by the rhPTH treatment in wild-type osteoblasts was suppressed in the DKO osteoblasts. Contrarily, cAMP accumulation in response to rhPTH was similar between cells of the two genotypes. These lines of results demonstrate the inhibitory role of the Gαq / PKCδ signal in the bone anabolic action of PTH, suggesting that suppression of the signal may lead to a novel treatment with PTH against osteoporosis.

085

MONOCYTES CONTROL MESENCHYMAL STEM CELL DIFFERENTATION TOWARDS OSTEOBLASTS

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Mesenchymal stem cells (MSC) are multipotent progenitors that can be induced in culture to form osteoblasts, adipocytes and chondrocytes. Coculturing MSC with peripheral blood mononuclear cells induced their differentiation towards osteoblasts as shown by alkaline phosphatase staining and the formation of bone nodules; this occurred in both control and osteogenic media. Purified populations of T cells, B cells and monocytes were isolated and cocultured with MSC revealing that monocytes were the cells responsible for this differentiation signal. To further dissect the monocyte population controlling differentiation, monocytes were sorted into CD14++16- and CD14+16+. The CD14+16+ monocytes, a minor population of monocytes in human peripheral blood, have been implicated in several inflammatory diseases; these cells are elevated in the both the joint and blood of rheumatoid arthritis (RA) patients. When cocultured with MSC, both populations of cells were able to induce MSC differentiation and this could be inhibited by the addition of IL-10 receptor neutralising antibodies. Interestingly, when monocytes were stimulated with LPS their activities differed greatly with the CD14++16- cells stimulating a vastly increased differentiation response whilst the CD14+16+ cells completely inhibited MSC differentiation. The CD14++16- population is known to produce IL-10 whilst the CD14+16+ population are the major producers of TNF and fail to make appreciable levels of IL-10. These findings have implications for the lack of bone repair in the RA joint. The presence of highly inflammatory CD14+16+ cells, combined with dysregulated cytokine production will prevent osteoblast formation whilst providing a suitable environment and precursors for excessive osteoclast formation thus exacerbating joint destruction.

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STRONTIUM RANELATE AMPLIFIES THE EFFECTS OF PTH BY IMPROVING THE INTRINSIC TISSUE QUALITY OF THE NEWLY FORMED BONE.

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Beside the effects of strontium ranelate (SR) on bone cellular activities, dissociating bone formation and resorption and resulting in a positive bone balance, SR is known to improve intrinsic bone tissue quality. The aims of this study are (i) to evaluate whether the association of SR with PTH or Alendronate can be synergistic and able to better prevent the decrease of bone strength in OVX rats than either drug administered alone and (ii) to investigate whether its potential additive effects are dependent on the in vivo stimulation of new bone formation. OVX rats were treated with a stimulator of bone formation (PTH 8ug/Kg*day,SC) or a treatment decreasing bone remodeling (Alendronate 18 µg/kg twice a week,SC) with or without strontium ranelate (625 mg/kg,PO). Six groups of 12 rats (SHAM, OVX, PTH, Alendronate, SR-PTH, SR-Alendronate) received treatments or respective vehicles for 8 weeks. At the end of the experiment, vertebrae were removed for biomechanics, [micro]CT and nanoindentation testing. PTH and Alendronate prevented the deleterious effect of OVX, with PTH increasing load compared to sham. This was associated with the preservation of bone volume in Alendronate treated animals and a significant increment under PTH. Combined therapy with SR resulted in a further significant increase of maximal load as compared with PTH treated rats only. The addition of SR improved intrinsic bone tissue quality in SR-PTH treated rats and corrected the impaired intrinsic tissue quality observed under PTH-treatment. Furthermore, it resulted in a further significant increment of trabecular thickness reflecting the positive influence of SR on bone balance. No effect was observed in the case of the association with Alendronate. Together, these data suggest that strontium ranelate maximizes the in vivo effect of PTH by improving

the intrinsic bone quality of the newly formed bone. The absence of synergistic effects when combined with Alendronate underlines the necessity of new bone formation.

	SHAM	OVX	PTH	Sr-PTH	Alendronate	Sr-Alendronate
Maximal Load (N)	323±93#	240±47	406±74#	474±79°#	357±112#	385±103#
BV/TV (%)	0.337±0.043	0.279±0.059	0.477±0.054#	0.496±0.040#	0.380±0.530#	0.398±0.061#
TbTh (mm)	0.098±0.001#	0.090±0.002	0.129±0.003#	0.135±0.002°#	0.100±0.001#	0.103±0.002#
Hardness (mPa)	398±82	369±111	335±109	419±99°#	432±120#	422±155

Values are means ±SD, significance of differences were evaluated using a Mann-Whitney test. # indicate a significant difference versus OVX and ° between PTH-SR and PTH

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VERTEBRAL FRACTURE RISK AND ALENDRONATE EFFECTS IN POSTMENOPAUSAL WOMEN ASSESSED BY CT-BASED NONLINEAR FINITE ELEMENT ANALYSIS

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CT-based nonlinear finite element analysis (FE) can accurately predict vertebral strength *ex vivo*. This study aimed to assess vertebral fracture risk and alendronate effects on osteoporosis *in vivo* using FE. Vertebral strength in 104 postmenopausal women (mean age, 71.3 years) was analyzed and the discriminatory power for vertebral fracture was assessed cross-sectionally. Alendronate effects were also prospectively assessed in 33 patients (mean age, 76.5 years) with postmenopausal osteoporosis who were treated with alendronate at a dose of 5 mg/day, compared with 8 women (mean age, 76.3 years) without any drug therapy for osteoporosis as controls. On the age and body weight adjusted logistic regression, vertebral strength by FE had stronger discriminatory power for vertebral fracture (OR per SD change: 6.71) than areal BMD (OR: 1.83) and volumetric BMD (OR: 3.57). The area under the receiver operator characteristic curve for vertebral strength was 0.822, which was significantly larger than that for areal BMD (area=0.713, p=0.0010), and volumetric BMD (area=0.767, p=0.0129). The optimal point for the vertebral fracture threshold was 1.95 kN, equivalent to 3.94 times body weight, with 75.9% sensitivity and 78.7% specificity. In the alendronate treatment group, vertebral strength significantly increased by 10.2% from baseline (p<0.0001) at 3 months. The correlation between percentage change from baseline at 3 months in vertebral strength and that in urinary NTx was low (r=0.295), indicating that vertebral strength does not incorporate effects on bone turnover. The correlation between percentage change at 12 months in vertebral strength and that in areal BMD was moderate (r=0.481). The minimum principal strain distribution showed that the area of high fracture risk decreased during alendronate therapy. At 1 year, the density of the inner cancellous bone increased by 8.3% (p=0.0013), while the density of the peri-cortical area increased by 13.6% (p=0.0004). In the untreated control group, none of the values showed significant changes. FE had higher discriminatory power for vertebral fracture than areal/volumetric BMD and detected alendronate effects at 3 months. Alendronate treatment was more effective in peri-cortical areas than in the inner trabecular compartment, which might be attributable to prolonged secondary mineralization and decreased cortical porosity. Alendronate altered density distribution, thereby improving minimum principal strain distribution and decreasing the area with high fracture risk, resulting in increased vertebral strength. Alterations in density distribution would be one of the factors detectable by FE but not areal/volumetric BMD.

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SEQUESTOSOME 1 MUTATIONS IN PAGET'S DISEASE OF BONE IN AUSTRALIA: PREVALENCE, GENOTYPE/PHENOTYPE CORRELATION AND A NOVEL NON-UBA DOMAIN MUTATION (P364S) ASSOCIATED WITH INCREASED NF-KB SIGNALLING WITHOUT LOSS OF UBIQUITIN-BINDING

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Previously reported *Sequestosome 1*(*SQSTM1*)/p62 gene mutations associated with Paget's disease of bone (PDB) cluster in, or cause deletion of the ubiquitin-associated (UBA) domain. The aims of this study were to examine the prevalence of SQSTM1 mutations in Australian patients, genotype/phenotype correlations and the functional consequences of a novel point mutation (P364S) located upstream of the UBA. Mutation screening of the *SQSTM1* gene was conducted on 49 kindreds with PDB. In addition, 194 subjects with apparently sporadic PDB were screened for the common P392L mutation by restriction enzyme digestion. HEK293 cells stably expressing receptor activator of NF-κB (RANK) were co-transfected with expression plasmids for SQSTM1 (wild type or mutant) or empty vector and a NF-κB luciferase reporter gene. GST-SQSTM1 (wild type and mutant) proteins were used in pull-down assays to compare monoubiquitin-binding ability. We identified *SQSTM1* mutations in 12 of 49 families screened (24.5%), comprising 9 families with the P392L mutation and one family each with the following mutations: K378X, 390X and a novel P364S mutation in exon 7, upstream of the UBA. The P392L mutation was found in 9 of 194 (4.6%) patients with sporadic disease. Haplotype analysis was performed on the sporadic P392L carriers, 8 individuals carried the mutation on the H2 haplotype ((916C-976G-2503T-2687G) while 1 carried the mutation on the H1 haplotype (916T-976A-2503C-2687T), providing further evidence for a proposed founder effect for this common mutation. Subjects with *SQSTM1* mutations had more extensive disease, but not earlier onset compared with subjects without mutations. In functional studies, the P364S mutation increased NF-κB activation compared with wild type SQSTM1, but unlike previously investigated mutations did not affect the ubiquitin-binding ability of SQSTM1. This suggests that increased NF-κB signalling, but not the impairment of ubiquitin-binding, may be essential in the pathogenesis of PDB associated with *SQSTM1* mutations.

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CLASS IA PHOSPHATIDYLINOSITOL 3-KINASES ARE INDISPENSABLE FOR OSTEOCLAST FUNCTION BY REGULATING CYTOSKELETAL ORGANIZATION AND CELL DEATH

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Phosphatidylinositol 3-kinases (PI3Ks) are a family of enzymes that phosphorylate phosphatidylinositol lipids at the 3' position. They are divided into 3 classes based on their structures and substrates. Class IA PI3Ks regulate signaling pathways downstream of tyrosine kinases such as c-Fms and Src. They consist of an adaptor subunit p85 (α and β), and a catalytic subunit p110 (α, β, δ). Previous studies have reported that PI3Ks play an important role in osteoclast activity by using specific inhibitors such as wortmannin although the exact role of the molecules in the skeletal tissue in vivo still remains unknown. To elucidate the role of class IA PI3Ks in osteoclasts, we generated osteoclast-specific p85α and β double knockout mice. Since conventional p85α knockout mice die within 3 weeks, we generated osteoclast-specific p85α knockout (p85α cKO) mice by crossing pik3r1 (p85α, p55α, p50α) flox mice and cathepsin K-Cre knockin mice. In p85α cKO mice, p85α expression was specifically reduced in osteoclasts but not in osteoblasts or other tissues. They were then crossed with pik3r2 (p85β) knockout mice to generate osteoclast-specific p85α&β double

knockout (p85 dKO) mice. Akt activation in response to macrophage colony-stimulating factor (M-CSF) stimulation was reduced in osteoclasts derived from p85α cKO mice, and almost completely abolished in p85 dKO osteoclasts. Radiological and histological analysis showed an increased bone mass in p85α cKO mice compared to control littermates with no statistical significance. While no abnormality was observed in the skeletal tissue of p85β KO mice, p85 dKO mice exhibited about 1.5 fold increase in bone mass compared to p85β-/- littermates ($p<0.05$). Eroded surface / bone surface was reduced by 50% while there was no significant difference in osteoclast number. Osteoclasts differentiated from p85α cKO and p85 dKO bone marrow cells showed impaired spreading and actin ring formation, and their bone-resorbing activity was remarkably suppressed. DKO OCs exhibited an increased cell death after cytokine withdrawal, and the proapoptotic phenotype was completely restored by M-CSF treatment, which activated Erk but not Akt in the cells. From these observations, we conclude that class IA PI3Ks are indispensable for osteoclast function by regulating cytoskeletal organization and cell death.

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DC-STAMP REGULATES BONE HOMEOSTASIS THROUGH CELL-CELL FUSION OF OSTEOCLASTS

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The balance between osteoclast and osteoblast activity is central for maintaining the integrity of bone homeostasis. Multinucleation by cell-cell fusion is a characteristic of osteoclasts. Recently, we have isolated DC-STAMP (Dendritic Cell Specific Transmenbrane Protein), a seven transmembrane protein, as an essential molecule for osteoclast cell-cell fusion, and demonstrated that osteoclasts in DC-STAMP knockout mice show complete lack of cell-cell fusion.(1) However, it is not yet characterized the role of osteclast cell-cell fusion via DC-STAMP in bone homeostasis. Here, we generated DC-STAMP transgenic mice (Tg) under the control of an actin (CAG) promoter to express DC-STAMP ubiquitously *in vivo*. DC-STAMP expression in the cells derived from DC-STAMP Tg mice was significantly upregulated, and the defects of osteoclast cell-cell fusion in DC-STAMP knockout mice was rescued *in vivo* and *in vitro* by crossing with DC-STAMP Tg mice. DC-STAMP Tg mice showed increased bone resorption with decreased osteoblastic activity and bone mass. Bone parameters such as bone volume per tissue volume and trabecular number were significantly downregulated in DC-STAMP Tg mice, whereas these parameters were significantly upregulated in DC-STAMP knockout mice. Bone resorption and formation are known to be regulated in a coupled manner, whereas DC-STAMP regulates bone homeostasis in an un-coupled manner. Indeed, DC-STAMP knockout mice exhibited impaired bone resorption and upregulation of bone formation by osteoblasts that do not express DC-STAMP, which led to increased bone mass. DC-STAMP expression was detected in various tissues of DC-STAMP Tg mice such as liver, muscle and brain, all of which do not express DC-STAMP physiologically in wild type mice. Interestingly, ectopic cell-cell fusion was not observed in liver and muscle, and the multinucleation of myotube was not stimulated by the forced expression of DC-STAMP in Tg mice. Thus, our results indicate that inhibition of a single molecule provides both decreased osteoclast activity and increased bone formation by osteoblasts, thereby increasing bone mass in an un-coupled and a tissue specific manner.

(1) Yagi M, Miyamoto T, et al., DC-STAMP is essential for cell-cell fusion in osteoclasts and foreign body giant cells. J Exp Med. 2005 202(3):345-51

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ROR2 SIGNALING ENHANCES OSTEOCLAST FORMATION IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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Wnt ligands activate β-catenin (β-cat)-dependent (canonical) and β-cat-independent (noncanonical) signaling pathways. Canonical Wnt signaling has been reported to suppress bone resorption via up-regulation of OPG expression and suppression of RANKL expression in osteoblasts (OB). However, the roles of noncanonical Wnt signaling in bone resorption are largely unknown. Using murine culture systems, we previously provided evidence for the stimulatory action of noncanonical Wnt signaling in osteoclastogenesis as follows: (1) Wnt5a, a noncanonical Wnt ligand, enhanced RANKL-induced osteoclast (OC) formation in cultures of bone marrow macrophages (BMMφ), OC precursors (OCP). (2) Wnt5a induced phosphorylation of c-Jun N-terminal kinase and protein kinase C but not accumulation of cytoplasmic β-cat in BMMφ. (3) Ror2, a co-receptor of Wnt5a, mediated the stimulatory effect of Wnt5a on RANKL-induced OC formation. To further clarify the role of noncanonical Wnt signaling in bone remodeling, we analyzed osteoclastogenesis in Ror2 and Wnt5a mutant mice. (1) $Ror2^{+/-}$ and $Ror2^{-/-}$ mice exhibited impaired osteoclastogenesis in the proximal femora at E18.5. (2) A similar defect in osteoclastogenesis was observed in $Wnt5a^{+/-}$ mice at E18.5. (3) OC formation induced by $1\alpha,25(OH)_2D_3$ was significantly lowered in cocultures of $Ror2^{-/-}$ OCP with WT OB and in cocultures of WT OCP with $Wnt5a^{-/-}$ OB. (4) No differences in expression of M-CSF, RANKL and OPG mRNAs were detected between Wnt5a mutant OB and WT OB treated with or without $1\alpha,25(OH)_2D_3$. (5) The impaired osteoclast formation in coculture with $Wnt5a^{-/-}$ OB was rescued by expression of Wnt5a in those OB, suggesting that Wnt5a produced by OB enhances osteoclastogenesis in the coculture. Wnt5a has been shown to be expressed in rheumatoid arthritis (RA) synovium. We then examined whether administration of GST-soluble Ror2 (sRor2), a decoy receptor of Wnt5a, to type ll collagen-induced RA model mice can prevent the bone destruction associated with RA. (6) Daily injection of sRor2 to those RA model mice prevented the joint destruction through the suppression of osteoclastogenesis. Collectively, these results suggest that Ror2 signaling stimulates osteoclastic differentiation of OCP in a cell-autonomous manner, and that noncanonical Wnt signaling is essentially involved in both physiological bone remodeling and pathological bone destruction associated with RA.

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ESSENTIAL ROLE OF SMALL GTPASE RAC1 DURING LIMB DEVELOPMENT

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Members of the Rho family including small GTPase Rac1 have been shown to play multiple roles in cell regulation, including regulation of cytoskeletal organization, cell migration, proliferation, and apoptosis. However, their tissue-specific roles *in vivo*, especially in mammalian limb development, remain largely unknown. In the present study, we employed a Cre-loxP system for limb bud mesenchyme-specific inactivation of the *Rac1* gene (Rac1 conditional knockout mice: Rac1 cKO mice) using *Prx1-Cre* transgenic mice, since *Rac1* null mice have embryonic lethal characteristics. Although most Rac1 cKO mice were viable at birth, 81% of the Rac1 cKO mice died within 3 weeks, probably due to incomplete fusion of the sternum. Detailed analyses of growth over the first 7 weeks of life demonstrated that Rac1 cKO mice were shorter in body length and had a lower body weight compared to the controls. Skeleton preparation of limbs from Rac1 cKO neonates stained with alcian blue and alizarin red confirmed that they had smaller skeletons than the controls. In addition, growth plates were severely disorganized and altered cell shape of chondrocytes was apparent in Rac1 cKO mice. Measurement of the length of different growth plate zones demonstrated that the both proliferative and hypertropic zones were shortened in Rac1 cKO mice. These phenotypes

are similar in cartilage-specific Rac1-deleteion mice using *Col2-Cre* transgenic mice. The most striking feature of the fore- and hind-limbs of Rac1 cKO mice was profound soft tissue syndactyly. Such soft tissue condition was not caused by fusion between the bones of the adjacent digits. To determine whether Rac1 cKO webbing was due to a defect in programmed cell death, TUNEL assays were performed on the controls and Rac1 cKO embryos at E12.5-E14.5, and demonstrated a significant reduction in the degree of interdigital cell death in Rac1 cKO limbs. Whole mount *in situ* hybridization analysis provided that expressions of *Bmp2*, *7* and *Msx1*, *2* were down-regulated in *Rac1* cKO mice, suggesting that insufficient expressions of these genes may be a root cause of the lack of interdigital cell death and associated syndactyly. Our findings demonstrated the essential role of Rac1 in limb development.

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PATHOPHYSIOLOGY OF BONE METASTASIS

T. Guise

To be provided

Abstract to be provided

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MANAGEMENT OF CANCER AND BONE: TRIALS AND TRIBULATIONS

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Recognition of the critical intermediary function of the osteoclast in the pathology of metastatic cancer cells involving bone led to clinical trials of adjuvant bone active agents such as bisphosphonates in patients with bone metastases, compared to purely cytotoxic approaches. Further research into the nature of the "vicious cycle" of bone destruction and enhancement of tumour growth is leading to the development of more specifically targeted bone active agents which may have fewer side-effects and toxicities than the bisphosphonates.

Hypercalcemia is now a less common condition in clinical practice, being easily managed by a variety of IV bisphosphonates. Prevention of the complications of bone metastases and associated bone disease in breast cancer and myeloma with bisphosphonates is now well established. No one bisphosphonate has been demonstrated in comparative trials to be more efficacious than another in pain relief or fracture prevention, although toxicities may differ. Most bisphosphonates (oral clodrononate and ibandronate, IV pamidronate and IV zoledronate) when given to ensure maximum compliance will reduce skeletal complications by around 30%.

In trials whose objective is the prevention of bone metastases, mixed results have been obtained. It is likely that a small proportion of patients have an inhibition of the growth of sub-clinical metastases. Prevention trials of clodronate, pamidronate and zoledronate in breast cancer and myeloma will be discussed including early trials of adjuvant oral clodronate, NSABP B34 (oral clodronate versus placebo) SWOG/NSABP/Intergroup (oral clodronate versus oral ibandronate and oral zoledronate) and ABCSG-12 (IV zoledronate) in breast cancer and MRC trial VI in myeloma. Observer variance in diagnosis of bone metastases is an emerging problem in these trials.

Investigation of clinical relationships between bone mineral density, markers of bone turnover, tumour markers and clinical outcome are yielding interesting results. Serum P1NP may be a useful marker of early bone metastasis activity. Serial urine Ntx may be useful in following symptomatic patients on bisphosphonates.

The problem of treatment induced bone loss (TIBL) in women with breast cancer receiving chemotherapy causing premature menopause or rapid reductions in circulating estrogen levels (as with aromatase inhibitor therapy) is quite significant. This can be ameliorated with bone mineral density monitoring and therapy.

Chronic toxicities of long-term bisphosphonate therapy are an increasing concern, especially osteonecrosis of the jaw and renal proximal convoluted tubular necrosis. The possibility of a “paradoxical” increased fragility of bone due to accumulation of unrepaired microfractures and increased mineralisation with chronic use of high potency agents at dosages used commonly in Oncology may become a problem. In this respect, there is no known way of eliminating bisphosphonates once they have been integrated into the bone matrix.

Newer targeted agents such as m-TOR and c-SRC antagonists, Cat-K inhibitors are of interest. Of particular current interest to oncologists is the humanised monoclonal antibody, denosumab, since it is targeted specifically to RANK-

ligand. Efficacy may be maximized while the elimination of the monoclonal antibody may reduce the likelihood of chronic toxicities -- or at least enhance the likelihood of recovery.

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A RANDOMIZED TRIAL OF BALLOON KYPHOPLASTY AND NONSURGICAL CARE FOR ACUTE VERTEBRAL COMPRESSION FRACTURE

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Background: Balloon kyphoplasty is a minimally invasive procedure performed in patients with painful vertebral fractures with the goal of reducing pain and improving quality of life. We performed a randomized trial to assess its efficacy and safety.

Methods: Patients with up to 3 acute vertebral fractures were randomly assigned to kyphoplasty (N=149) or nonsurgical care (N=151). The intervention was not blinded. The primary outcome was the difference in the SF-36 physical component summary at one month. Quality of life measurements and spine radiographs were assessed through twelve months.

Results: Subjects randomized to kyphoplasty had greater improvement than controls in the SF-36 physical component summary (difference 5.2 points on a 0-100 scale; 95% confidence interval [CI], 2.9−7.4; p<0.001) at one month; the difference was 1.5 (95%CI, −0.8−3.8; p=0.2) at twelve months. Kyphoplasty improved quality of life by the 1-point EuroQol questionnaire at one (0.18 points; 95%CI, 0.08−0.28; p<0.001) and twelve (0.12; 95%CI, 0.01−0.22; p=0.025) months and back function by the 24-point Roland-Morris scale at one (4.0 points; 95%CI, 2.6−5.5; p<0.001) and twelve (2.6; 95%CI, 1.0−4.1; p=0.001) months. Kyphoplasty patients reported fewer days with limited activity, less back pain, and less use of analgesics or walking aids. Two serious adverse events, a soft tissue hematoma and a post-operative urinary tract infection, were attributed to kyphoplasty. The frequency of new vertebral fractures did not differ significantly between kyphoplasty (33.0 %) and nonsurgical (25.3%) groups (7.7% difference; 95%CI -4.5−20.0; p=0.22) . Two-year data will be available prior to the meeting and will be presented.

Conclusion: Among patients with acute vertebral fractures, balloon kyphoplasty improved quality of life, reduced back pain and disability and decreased pain medication and walking aid usage. Most of these improvements last at least one year without increasing adverse events or new vertebral fractures.

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ENZYME REPLACEMENT THERAPY (ERT) FOR MURINE HYPOPHOSPHATASIA

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Hypophosphatasia (HPP) is the inborn-error-of-metabolism that features rickets or osteomalacia due to loss-of-function mutation(s) within the gene that encodes the tissue-nonspecific isozyme of alkaline phosphatase (TNALP). Consequently, natural substrates for this ectoenzyme accumulate extracellulary including inorganic pyrophosphate (PPi), an inhibitor of mineralization, and pyridoxal 5'-phosphate (PLP), a cofactor form of vitamin B6. Babies with the infantile form of HPP often die with severe rickets and sometimes hypercalcemia and vitamin B6-responsive seizures.

There is no established medical treatment. We bio-engineered human TNALP with a C-terminus extended by the Fc region of human IgG for one-step purification, and a deca-aspartate sequence (D10) for targeting to mineralizing tissue (sALP-FcD10). TNALP null mice (Akp2-/-), an excellent model for infantile HPP, were treated from birth using sALP-FcD10. Short-term and long-term efficacy studies consisted of once daily s.c. injections of 1, 2, or 8.2 mg/kg sALP-FcD10 for 15, 19, and 15 or 52 days, respectively. We evaluated survival and growth rates, circulating levels of sALP-FcD10 activity, calcium, PPi, and pyridoxal, as well as skeletal and dental manifestations using radiography, μCT, and histomorphometry. Akp2-/- mice receiving high-dose sALP-FcD10 grew normally and appeared well without skeletal or dental disease or epilepsy. Plasma calcium, PPi, and pyridoxal concentrations remained in their normal ranges. We found no evidence of significant skeletal or dental disease and concluded that ERT with this bone-targeted form of TNALP prevents all the manifestations of infantile HPP (Millán et al., JBMR 23: 777-787, 2008). Subsequent dose response studies yielded a positive correlation between administered dose and 75% survival and mineralization improvement in the hind limbs. Longer dosing intervals with bone-targeted TNALP can be used to prevent the appearance of the mineralization defects and improve survival in the Akp2-/- mouse model of HPP. We also assessed rescuing overt disease by starting treatments at day 12 or 15 of life when the clinical manifestations of HPP were evident. ERT could also rescue Akp2-/- mice with advanced disease. Dosing less frequently than daily could also increase survival. Clinical trials administering bone-targeted TNALP to infants with HPP are now underway.

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ASSOCIATION BETWEEN HYPERGLYCEMIA AND FRACTURE RISK IN NON-DIABETIC MIDDLE-AGED AND OLDER AUSTRALIANS: A NATIONAL, POPULATION BASED PROSPECTIVE STUDY (AUSDIAB).

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Purpose: The relationship between type 2 diabetes mellitus (DM) and fracture risk is controversial; even less is known about the relationship between impaired glucose tolerance and fracture risk. The primary aim of this study was to examine the association between plasma glucose (PG) and insulin levels and fracture risk in non-diabetic middle-aged and older Australians. The secondary aim was to assess whether fracture risk was increased in people with pre-diabetes [impaired glucose tolerance (IGT) or impaired fasting glucose (IFG)]. Methods: This national, population-based prospective study undertaken in 1999/2000 (AusDiab) with a follow-up of 5 years included 6,255 men and women aged ≥40 years (mean ± SD; 56.2 ± 11.0) with normal glycemia (NGT, n=4,855) and pre-diabetes (IFG and IGT, n=1400) determined from an OGTT (1999 WHO criteria) at baseline. People with diagnosed type 2 DM were excluded. Non-traumatic clinical fractures (eg. fall from a standing height) were self reported at follow-up and used to calculate incident fracture data. Results: Overall, 318 (5.1%) of the participants suffered at least one non-traumatic fracture (women 7.4%; men 2.2%). After adjusting for age, BMI and smoking, having a 2-h PG ≥8.29 mmol/L (highest quartile) was significantly associated with a decreased risk of fracture in women [OR 0.62 (95%CI 0.42, 0.92)], but not men (see Figure). These results remained unchanged after adjusting for fasting insulin, physical activity, or previous history of low trauma fracture. Similar results were observed when the analyses were repeated with 2-h PG as a continuous variable. However, no significant associations were found for fracture risk and quartiles of fasting PG or insulin. Logistic regression analysis also revealed that compared with women with NGT, there was a trend for those with pre-diabetes to have a reduced fracture risk after adjusting for age, BMI and smoking [OR 0.72 (95% CI 0.51, 1.01)]. Conclusion: Increased 2-h PG levels and pre-diabetes appeared to be protective against non-traumatic clinical fractures in non-diabetic middle-aged and older women, and these findings were independent of fasting insulin levels.

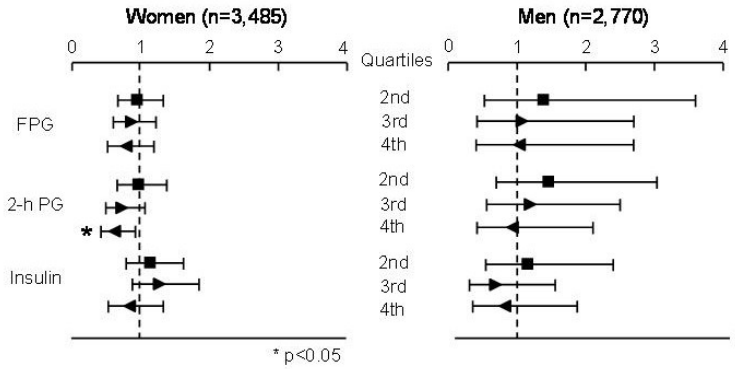

Fig. OR (95% CI) for incident fractures according to quartiles of FPG, 2h-PG and fasting insulin.

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EFFECTS OF RISEDRONATE ON BONE MARROW ADIPOCYTES IN POSTMENOPAUSAL WOMEN

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Introduction: Age-related bone loss is associated with high levels of adipogenesis within the bone marrow. Since osteoblasts and adipocytes share a common marrow stromal cell precursor, it is possible that with aging, there is a preferential "switch" in commitment of this precursor to the adipocyte over the osteoblast lineage. Previously, we have reported a potential anabolic effect of bisphosphonates through the stimulation of osteoblastogenesis and inhibition of adipogenesis in vitro (1). In the current study, we tested the hypothesis that the effect of bisphosphonates on marrow adipogenesis in vitro is also present in vivo. Methods: We analyzed transiliac bone biopsies from a randomized, placebo-controlled clinical trial that evaluated the effects of risedronate treatment 5 mg/day on vertebral and non-vertebral fractures in women with postmenopausal osteoporosis. Paired bone biopsies were obtained from a subset of patients at baseline and after treatment with placebo or risedronate for 3 years (placebo, n=18, risedronate, n=14). Biopsies were stained with toluidine blue and hematoxylin/eosin. Adipocyte volume/tissue volume (AV/TV) adipocyte number (Ad#) and mean adipocyte size were quantified. Results: In the placebo group AV/TV and Ad# significantly increased (15%). In contrast, there were significant decreases in these parameters (28%) in the risedronate group ($p<0.01$). In addition, mean adipocyte size was significantly reduced (25%) in the risedronate group ($p<0.01$) at 3 years. Conclusion: Risedronate reduces marrow adipogenesis in postmenopausal women, independently of its effect on bone mass. These findings are the first demonstration of an effect of bisphosphonates on marrow fat in humans in vivo. This effect may contribute to the beneficial effect of bisphosphonates on bone mass.

(1) Alendronate has an anabolic effect on bone through the differentiation of mesenchymal stem cells. Duque G, Rivas D. J Bone Miner Res. 2007, 22:1603-11

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SKELETAL DYSPLASIA AND LETHALITY IN MICE LACKING THE MID-REGION, NUCLEAR LOCALIZATION SIGNAL AND C-TERMINUS OF PARATHYROID HORMONE-RELATED PROTEIN

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Parathyroid hormone (PTH)-related protein (PTHrP) is a pleiotropic factor with multiple functions that include regulation of morphogenesis, cell proliferation, differentiation, and apoptosis, as well as transplacental calcium transport. PTHrP exerts many of its skeletal actions by binding to the PTH-1 receptor (PTH1R) via its N-terminus. A number of *in vitro* studies suggest that some of the effects of PTHrP on cell function are not mediated by the N-terminus, but rather by the mid-region, a nuclear localization sequence (NLS), and the C-terminus. The aim of this study was to determine whether the mid-region, NLS, and C-terminus of PTHrP have *in vivo* functions on skeletal development.

We generated a knock-in mouse lacking PTHrP-(67-137), which encompasses these three domains (*PTHrPΔ/Δ*). The presence of the mRNA and protein for *PTHrPΔ/Δ* was demonstrated in fetal and newborn tissues. *PTHrPΔ/Δ* pups had an abnormal phenotype characterized by a domed head and a short muzzle (craniodysplasia), short limbs (chondrodysplasia), and a hunched back (kyphosis) when compared to *PTHrPΔ/+* and *PTHrP+/+* (controls). *PTHrPΔ/Δ* pups failed to thrive and most of them died by day 5 (few lived up to 3 weeks). MicroCT and histomorphometry revealed decreased bone mass, trabecular thickness, trabecular number, and cortical bone thickness. *PTHrPΔ/Δ* pups had craniofacial dysplasia with short maxillae and mandible, and decreased calvarial bone density. Dental eruption was delayed and incisors were discolored and deformed. Long bones had shorter growth plates, decreased chondrocyte and osteoblast number, increased TRAP+ cells and TUNEL labeling, and decreased bone marrow cellularity. *Runx2, Ocn, Sox9, Crtl1,* and *ß-catenin* were underexpressed while *Ffg3, Ihh, Il-6,* and *P63* were overexpressed. Hematology and blood chemistry revealed hypocalcemia, hypoglycemia, hypolipemia, hypoinsulinemia, and leukopenia. Serum osteocalcin was decreased in mutants, but no differences were found in PTH, PTHrP, and TRAP 5b concentrations.

These findings support our hypothesis that the mid-region, NLS, and C-terminus of PTHrP are essential for skeletal ontogenesis and viability. In addition, PTHrP mediates interactions between the hematopoietic and skeletal compartments, as well as participates in energy metabolism.

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INHIBITING GLYCOGEN SYNTHASE KINASE-3 (GSK-3) PREVENTS THE DEVELOPMENT OF MYELOMA BONE DISEASE.

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Multiple myeloma is a B-cell malignancy characterised by the growth of tumour cells in the bone marrow and the development of osteolytic bone disease. This is mediated by increased osteoclastic bone resorption and a suppression of bone formation. Regulators of the bone resorption have been identified; however, understanding of the mechanism responsible for suppressing bone formation is poor. Recently, the Wnt pathway has been implicated in regulating bone formation and myeloma cells produce soluble antagonists of Wnt signaling, which suppress bone formation. GSK-3 phosphorylates beta-catenin and blocks Wnt signaling. Thus, inhibiting this enzyme may remove myeloma-induced suppression of osteoblastic bone formation. In this study we investigated whether blocking GSK-3, with AR28, a potent GSK-3 inhibitor, prevents myeloma-induced suppression of bone formation and the development of osteolytic disease in the 5T2MM model of myeloma. Injection of C57Bl/KaLwRij mice with 5T2MM myeloma cells suppressed osteoblast numbers ($p<0.05$) and promoted formation of osteolytic lesions ($p<0.05$). Treatment of C57Bl/KaLwRij mice with AR28 (15µmol/kg, twice daily, 4 weeks) increased cancellous bone volume. Treatment of C57Bl/KaLwRij mice bearing 5T2MM cells with AR28 (15µmol/kg or 45µmol/kg) increased osteoblast numbers ($p<0.05$) and osteoblast perimeter ($p<0.05$), and prevented the development of osteolytic lesions ($p<0.05$). Treatment had no effect on osteoclast numbers. AR28 also had no effect on serum paraprotein, a marker of whole animal tumour burden, or spleen weight, a site of extramedullary proliferation. However, myeloma burden in bone was decreased by 40%. These

data suggest that blocking GSK3 with AR28 prevents myeloma suppression of bone formation and blocks the development of osteolytic bone disease, independent of effects on osteoclast numbers. This suggests that GSK-3 inhibitors may have therapeutic potential in patients with multiple myeloma

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APOMAB, A FULLY HUMAN AGONISTIC DR5 MONOCLONAL ANTIBODY INHIBITS TUMOUR GROWTH AND OSTEOLYSIS IN MURINE MODELS OF BREAST CANCER DEVELOPMENT AND PROGRESSION

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Apomab, a fully human agonistic monoclonal antibody binds specifically to Apo2L/TRAIL death receptor DR5 and triggers apoptosis through activation of the extrinsic apoptotic signaling pathway. In this study we assessed the cytotoxic affect and signaling of Apomab *in vitro* and evaluated its antitumour activity in murine models of breast cancer development and progression at both the orthotopic site and in bone. MDA-MB-231-TXSA cells, tagged with a triple reporter gene construct (NES-HSV-tk/GFP/Luc), were transplanted directly into the mammary fat pad or into the tibial marrow cavity of nude mice. Tumour progression with and without Apomab treatment was monitored in live animals and in real time using D-luciferin-induced bioluminescence imaging, whereas the development of breast cancer-induced osteolysis was measured using high resolution micro-computer tomography and histology. *In vitro*, Apomab treatment resulted in a dose-dependent increase in apoptosis in four of the nine well-established breast cancer cell lines tested. This was associated with processing and activation of caspases 8, 10, 9 and 3, over time and was concomitant with cleavage of the apoptosis target proteins Bid and PARP. Importantly, Apomab was without effect on normal human primary osteoblasts, fibroblasts or mammary epithelial cells in culture. *In vivo*, Apomab treatment resulted in complete regression of well-advanced tumours (1000 mm^3) within the mammary fat pad and with no evidence of recurrence. Animals transplanted with cancer cells directly into their tibiae and left untreated, all developed large lesions that invaded the marrow cavity, eroded the cortical bone and tumour growth extended into the surrounding soft tissues. In contrast, Apomab treated mice showed remarkable inhibition of tumour growth within the marrow cavity and complete protection from breast cancer induced osteolysis. These results suggest that Apomab represents a potent immunotherapeutic agent with strong activity against the development and progression of breast cancer and highlights the need to clinically evaluate Apomab in patients with primary and metastatic disease.

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STAT3 INDUCES OSTEOBLASTIC BONE METASTASES THROUGH UP-REGULATING THE EXPRESSION OF LEPTIN AND LEPTIN RECEPTORS IN THE LNCAP HUMAN PROSTATE CANCER

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Prostate cancer (PCa) is one of the leading threats to men's health in the world. It frequently spreads to bone to cause osteoblastic bone metastases. However, the mechanism of osteoblastic bone metastases is unclear. Signal transducers and activators of transcription 3 (STAT3), which is one of the oncogenic signaling molecules, is constitutively activated in human PCa and known to be involved in the promotion of PCa aggressiveness. Moreover, STAT3 is reported to stimulate osteoblastogenesis. Collectively, we reasoned that STAT3 plays a role in the development of osteoblastic bone metastases in PCa. We introduced a constitutive-active STAT3 cDNA into the LNCaP human PCa cells (LNCaP/caSTAT3 cells). LNCaP/caSTAT3 cells showed increased proliferation and anchorage-independent growth and tumorigenicity compared with LNCaP/EV cells. Histological examination revealed that LNCaP/caSTAT3 cells initially developed osteolytic, followed by mixed and finally osteoblastic metastases following intracardiac inoculation, whereas LNCaP/EV failed to develop bone metastases. MicroArray analysis revealed that LNCaP/caSTAT3 cells showed increased expression of leptin receptors and western analysis displayed not only increased leptin receptors but also leptin in LNCaP/caSTAT3 cells. Obesity is a risk factor of PCa. Circulating levels of

leptin, which is an *ob* gene product, are elevated in aggressive PCa. Furthermore, leptin is shown to stimulate bone formation. Accordingly, we focused on studying the role of leptin and leptin receptors in osteoblastic bone metastases in LNCaP/caSTAT3 cells. Immunohistochemical examination showed strong leptin and leptin receptor expression in osteoblastic bone metastases of LNCaP/caSTAT3. Leptin antagonist significantly decreased LNCaP/caSTAT3 cell proliferation, suggesting leptin is an autocrine growth-stimulatory factor. LNCaP/caSTAT3 conditioned medium (CM) stimulated the differentiation and calcification in human osteoblastic cells (HAOB). These effects of CM were blocked by leptin antagonist . Recombinant leptin also stimulated osteoblastic differentiation. Finally, we found leptin promoted osteoblastic differentiation via activation of STAT3 and ERK. In conclusion, we established an animal model of osteoblastic bone metastases of human PCa. Our results using this model suggest STAT3/leptin axis plays an important role in the development of osteoblastic bone metastasis of PCa. They also provide a molecular basis of the relationship between obesity and the development of PCa.

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BLOCKADE OF THE ALK1 RECEPTOR REDUCES TUMOUR MICROVESSEL DENSITY AND PREVENTS THE FORMATION OF OSTEOLYTIC BONE DISEASE IN THE 5T2MM MURINE MODEL OF MYELOMA

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Myeloma is a malignancy of differentiated B-lymphocytes. Myeloma growth in bone is associated with increased angiogenesis and the development of osteolytic lesions. Loss of function mutations in activin receptor-like kinase 1 (ALK1), a type 1 TGF-β superfamily receptor, result in vascular defects. Recently, blockade of ALK1 has been shown to inhibit angiogenesis. In the present study we sought to investigate the effect of blocking signalling through ALK1 using a soluble receptor comprised of the extra-cellular domain of ALK1 fused to the Fc portion of a mouse IgG immunoglobulin (RAP-041). C57/BLK/KalWrj mice were injected with 5T2MM murine myeloma cells and treated with RAP-041 (10mg/kg/ twice weekly / intra-peritoneal) or vehicle from the time of tumour cell injection (n=10/group). Additionally, naïve non tumour bearing mice were analysed as controls (n=10/group). Mice were sacrificed at week 12. Microvessel density was measured on histological sections. Bone volume and osteolytic lesions were analysed using microCT. Tumour burden, osteoblast and osteoclast number were measured using histomorphometry. Microvessel density was increased in mice bearing 5T2MM cells compared with naïve controls ($p<0.001$). Treatment with RAP-041 reduced microvessel density ($p<0.001$). Trabecular bone volume was reduced in mice bearing 5T2MM cells ($p<0.05$). This reduction was completely reversed in 5T2MM bearing mice treated with RAP-041 ($p<0.05$). Analysis of activated osteoclast number revealed significantly increased numbers of activated osteoclasts in 5T2MM bearing mice ($p<0.05$). Treatment with RAP-041 reduced the number of activated osteoclasts ($p<0.05$). Furthermore, treatment with RAP-041 reduced the number of osteolytic bone lesions seen in 5T2MM bearing mice compared with vehicle treated mice. No significant differences in osteoblast number or tumour load were observed in RAP-041 treated mice bearing 5T2MM cells compared with vehicle treated mice. These data suggest that blockade of ALK1 signaling in a tumor model reduces angiogenesis, osteoclast activation and prevents the development of osteolytic bone lesions.

OSTEOSARCOMA METASTASIS: EVIDENCE FOR ACP5 DOWNREGULATION AND OSTEOCLAST INVOLVEMENT

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Osteosarcoma (OS) is the most common primary bone tumour in the pediatric age group. Pulmonary metastasis, which can be present in up to 50% of cases, is the major fatal complication of osteosarcoma. While the long-term survival of patients without metastasis is 65-70%, the long-term survival of patients who develop metastasis is only 10-20%. Our gene expression profiling study of primary osteosarcoma biopsies identified tartrate-resistant acid phosphatase 5 (ACP5, TRAP), a marker of osteoclasts, which at the time of diagnosis, predicts with 93% accuracy those patients in our group that will progress to lung metastasis. These results have been confirmed by immunohistochemistry in the original patient biopsies. Furthermore, within the metastatic patient group, the time for the development of detectable metastasis correlates directly with the level of ACP5 expression. We have also shown that the loss of ACP5 is associated with a loss of osteoclasts, and that *in vitro*, this loss of osteoclasts correlates with enhanced osteosarcoma cell migration. We have used nine different osteosarcoma cell lines to set up an orthotopic mouse model in which manipulation of osteoclast number and activity, with zoledronic acid or with a low calcium diet, is able to modulate the tumorigenicity and metastatic potential of osteosarcoma. Our findings suggest that the loss of osteoclasts and concomitant downregulation of ACP5 may contribute to OS metastasis, and that modulators of osteoclast number and activity may have a significant impact in patients at risk of metastasis.

POSTERS
Category 1.
Systemic and Local Regulation of Skeletal Metabolism

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ORAL STRONTIUM RANELATE TREATMENT MARKEDLY IMPROVES IMPLANT OSSEOINTEGRATION.

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The employment of metallic implantation into bone is frequently utilized in orthopaedics and dentistry all the more with the aging population. The process of metallic implant osseointegration begins with a phase of bone resorption around the implant, with simultaneous bleeding and inflammation, and is followed by a phase of bone formation. Strontium ranelate, efficacious in the treatment of osteoporosis, has been shown to inhibit bone resorption, to positively influence bone formation and to improve bone material quality. Whether strontium ranelate treatment improves titanium implant osseointegration is not known. We measured the resistance to pull-out of 1 mm diameter titanium rods implanted into the proximal tibia of 6 month-old female rats. The titanium implants, sandblasted and acid-etched along the surface of non threaded part, were inserted into both tibias and the rats received strontium ranelate (625 mg/kg, 5/7 days, n=15) or alendronate (18 microg/kg/d, 2/7 days, n=15, positive control) or vehicles (n=15) for 8 weeks. The tibias were then removed for microtomographic histomorphometry and resistance to implant pull-out was tested by recording the maximal force necessary to completely loosen the implant. In a subgroup of samples, nanoindentation (intrinsic bone tissue quality in the vicinity of the implant) and histomorphometry were performed. All results are expressed as means±SEM (n). Significance of differences were evaluated by analysis of variance (* $p<0.05$ versus control) .

	Control	Strontium ranelate	Alendronate
Pull out Force (N)	32.52±3.79 (15)	43.54±3.03 * (15)	48.40±3.05 * (15)
Hardness (mPa) trabecular bone	624.8±21.6 (7)	721.7±27.6 * (7)	654.8±23.3 (7)
Hardness (mPa) cortical bone	837.3±24.5 (7)	918.5±30.54 * (7)	822.8±25.6 (7)

Both strontium ranelate and alendronate improved implant osseointegration as compared to the controls. This was associated with specific improvements of the determinants of pull-out force. Whereas both treatments positively influenced microarchitecture, intrinsic bone tissue quality in the vicinity of the implant was improved in rats treated with strontium ranelate but not in rats treated with alendronate. Furthermore, dynamic histomorphometry demonstrated tetracycline labelling along the implant in rats treated with strontium ranelate, but not in those rats treated with alendronate, compatible with the hypothesize that strontium ranelate may induce bony ingrowths into the sandblasted and acid-etched surfaces of the implants . The dose of strontium ranelate used, 625 mg/kg correlates to with human therapeutic exposure. In conclusion, whilst both treatments induce a similar pull-out force, only strontium ranelate improves implant osseointegration by positively altering formation of bone and the intrinsic bone material quality in the vicinity of the implant.

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TNF-RELATED WEAK INDUCER OF APOPTOSIS (TWEAK) AND SCLEROSTIN BOTH SIGNAL VIA ERK-1/2 PHOSPHORYLATION IN HUMAN OSTEOBLASTS

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Sclerostin is the product of the *SOST* gene, mutations in which cause diseases with high bone mass in humans as does deletion of *SOST* in mice, indicating that this molecule has a key role in the regulation of bone mass. The mechanism of action of sclerostin is not fully elucidated but appears to at least behave as an atypical inhibitor of both the BMP and Wnt signalling pathways. One activity of sclerostin appears to be similar to DKK1, to bind to and inhibit LRP5/6. We have recently shown that TWEAK, alone and together with TNF, induced the expression at both the mRNA and protein levels of sclerostin and that this was dependent on activation of the ERK-1/2 and JNK mitogen activated

protein kinase (MAPK) pathways (see accompanying abstract by Wijenayaka *et al*.). In order to study the downstream effects of sclerostin on human osteoblasts and compare them to those of TWEAK, we treated human primary osteoblasts (NHBC) with either recombinant human sclerostin or TWEAK. Consistent with both sclerostin and TWEAK behaving as inhibitors of osteoblast differentiation, both suppressed RUNX2 and OCN mRNA expression. Unexpectedly, TWEAK, TNF and sclerostin treatment of NHBC similarly altered levels of phosphorylated and total GSK3β, as well as active (hypophosphorylated) and total levels of β-catenin, implying that the Wnt signaling pathway was activated by all three stimuli. Sclerostin also rapidly activated ERK-1/2 MAPK signaling. Our results suggest that sclerostin interacts with the Wnt signalling pathways in a more complex fashion than previously thought and suggests that more study is required to elucidate the precise mode of action of this important regulator of bone mass.

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DIFFERENTIAL SKELETAL RESPONSES OF Y1 AND Y2 RECEPTORS TO CHRONIC STRESS

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Neuropeptide Y (NPY) has anxiolytic actions, attenuating the psychological response to stress. This study was designed to investigate if this also extends to bone and if so, identify the underlying mechanism.

The skeletal effects of NPY are known to involve Y1 and Y2 receptor signaling, thus stress responses were examined in the bones of $Y1^{-/-}$ and $Y2^{-/-}$ mice and compared to wild type and $NPY^{-/-}$. Mice under a mixed cold and restraint stress protocol for 6 weeks were collected at 16 weeks.

Chronic stress induced mild bone loss in wild type mice (13%, ns), with a significant loss of trabecular number (Tb.N, /mm) (3.8 ±0.2 vs 2.9 ±0.1, $p<0.005$). $NPY^{-/-}$ mice lost more cancellous bone volume (16.7% ±1.5 vs 11.6 ±1.7, $p<0.05$), again with reduced Tb.N. Stress reduced mineral apposition rate (MAR, μm/d) in *NPY-/-* (2.8 ±0.1 vs 2.2 ±0.1, $p<0.0001$), with no change in osteoclast indices. $Y2^{-/-}$ mice also lost cancellous bone (12.5 % ±0.8 vs 8.7 ±1.7, $p<0.05$) with reduced Tb.N and MAR (2.5 ±0.2 vs 1.9 ±0.1, $p<0.05$). $Y1^{-/-}$ mice displayed an opposing response to stress. Trabecular thickness was greater (30.9 μm ±1.5 vs 38.7 ±2.3, $p<0.02$), with reduced osteoclast surface (17.4% ±1.3 vs 12.7 ±1.1, $p<0.02$), increased MAR (2.0 ±0.1 vs 2.7 ±0.1, $p<0.0001$) and mineralizing surface (2.8% ±0.1 vs 2.2 ±0.1, $p<0.0001$).

Stress increased corticosterone in all genotypes but to different extents compared to controls. In stressed groups, $NPY^{-/-}$ had increased levels (315 ng/ml ±68) while $Y1^{-/-}$ mice had reduced levels (57 ±9). Despite a greater stress response, $Y2^{-/-}$ mice had similar corticosterone levels to wild type (*Y2-/-*116 ±22 vs wt 103 ±11).

In summary, NPY appears to be critical to maintaining osteoblast activity during chronic stress, via Y2 receptor signaling, with Y1 playing a counter-regulatory role. This protective effect in part involves suppression of corticosterone production. Modulation of NPY signaling may enable protection of skeletal tissue from the adverse effects of glucocorticoid excess.

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THE DIFFERENTIAL DISTRIBUTION IN VIVO OF FLUORESCENTLY-LABELED BISPHOSPHONATE ANALOGUES WITH DIFFERENT MINERAL AFFINITY TO BONE SURFACES.

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Background: Clinical evidence suggests that bisphosphonates (BPs) differ in their ability to reduce vertebral and nonvertebral fractures. While many produce similar reductions in vertebral fracture rates, there is greater disparity in

the speed and extent of reduction of nonvertebral fractures. We have proposed that different mineral affinity characteristics of BPs may lead to differential skeletal distribution, which could help to explain these diverse fracture results. *Methods*: We dosed 2-month and 8-month old male rats – subcutaneously and simultaneously – using fluorescently tagged BP analogues with contrasting mineral affinities (the higher affinity risedronate-fluorescein = Green, and a lower affinity phosphonocarboxylate analog, 3-PEHPC- rhodamine = Red), compared to no BP label. Fluorescent labels in bone and growth plate cartilage in proximal tibia, and bone, incisor dentine and molar cementum in mandible were studied 24h and one week after administration using highest resolution confocal fluorescence (Green and Red) in the immediate sub-surface of diamond sawn sections or polished PMMA embedded blocks. Focus level was controlled by using reflected light in the Blue channel. Mineral content was determined by 20kV backscattered electron SEM imaging of identical block fields. *Results*: Differences in mineral affinity affected both the distribution of BPs within calcified tissues and the depth of penetration. Specific findings: 1. Both compounds were found at sites of resorption. 2. There are areas of co-localization as well as Red or Green only on both resorbing and forming surfaces. Green label dominated mineralizing fronts active at administration and Red labels those that commenced after (including within 24h of) administration. Green labels dominated resorbed surfaces with no new matrix deposition. 3. Red diffused deeper into mineralized tissue than Green at all sites (resting, forming and resorbed). Red was observed in osteocytic lacunar walls deeper within bone – and deeper within the lacunar walls – than Green. 4. Neither compound blocked the progress of mineralization nor affected the level of mineralization. *Conclusion*: Differences in mineral affinity of bisphosphonate analogs appear to influence the distribution on (and depth of penetration into) bone surfaces in vivo, and therefore the relative amounts of these drugs, throughout the skeleton.

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THE EFFECT OF REPETITIVE LOADING ON BONE MASS AND GEOMETRY DURING GROWTH IS SITE-AND GENDER-SPECIFIC

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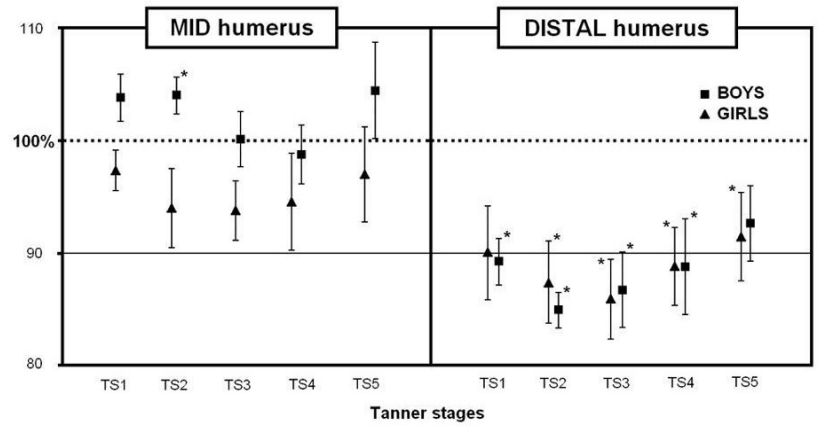
Aim. To assess the surface- and site-specific response of cortical bone to loading in boys and girls during pre-, peri- and post-pubertal years.

Methods. Eighty competitive tennis players (44 boys), mean age 13.7 years (9.2-18.9 years) had their dominant and nondominant humeri scanned by MRI. Total bone area (ToA), medullary area (MedA) and cortical bone area (CoA) were determined at the mid (40-50%) and distal humerus (60-70%). Humeral bone mass (BMC) was derived from the whole-body DXA scan. Pubertal status was self-assessed using Tanner stages (TS). All boys and girls started playing at the same age (7 years) and had similar training volume (14.0 hours/week).

Results. In boys and girls, BMC, ToA and CoA were greater on the dominant relative to non-dominant side from TS1 to TS5 (+5.9-36.9%, $p<0.05$). Side-to-side differences in BMC were greatest in TS3 boys (+32%, $p<0.0001$) and TS1 girls (+25.5%, $p<0.05$). Periosteal expansion (increase in ToA) was similar between the mid and distal humerus (boys: +12.8% and 14.0%; girls: +9.2% and 9.5%, respectively). In contrast, the endosteal surface showed a different response between the two sites ($p<0.0001$): there were little or no side-to-side differences in MedA at the mid humerus, whereas medullary contraction was observed at the distal humerus in both sexes (figure), leading to a greater increase in CoA at that site ($p<0.007$). The side-to-side difference in ToA and MedA at the distal humerus (periosteal expansion and medullary contraction) explained 50% (boys) and 58% (girls) of the variance of the extra BMC laid down on the dominant side ($p<0.001$).

Figure 1. Size of the medullary cavity of the dominant humeral shaft in tennis players (in % of the nondominant side)



* Significant side-to-side difference (p<0.05). Bars indicate S.E.

Conclusion. Geometrical adaptations, which explained a large part of the variance in the loading-induced bone accretion, depend on gender, pubertal stage and skeletal site. Extrapolating observations made at a particular skeletal site to the entire skeleton or the entire bone must be considered with caution.

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PHYSICAL FITNESS IS ASSOCIATED WITH GREATER BONE STRENGTH IN PREPUBERTAL CHILDREN, IRRESPECTIVE OF THEIR BODY WEIGHT

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Aim. To investigate the relationship between physical fitness and bone strength in overweight and normal-weight children.

Methods. Bone mass (BMC) and geometry (total area, ToA) were measured by pQCT at the radius and tibia (4% and 66%) in 598 children (296 boys), aged 7-10 years. Bone strength was estimated at the 4% (BSI) and 66% sites (polar SSI). Muscle and fat cross-sectional areas were measured by pQCT at the 66% sites and whole-body lean mass and fat mass were measured by DXA. Fitness was evaluated with a vertical jump (explosive power), 20m shuttle run (aerobic fitness) and holding the prone plank position (core strength).

Results. Overweight children (BMI>equivalent to 25 kg/m^2 in adults; 22% of the sample) were heavier (+3SD) and fatter (+2.7SD) but also taller (+0.6SD) and more muscular (+1.3SD) than normal-weight peers ($p<0.0001$). They also had greater bone parameters (+0.5-1.3SD, $p<0.001$). As expected, normal-weight children performed better in all fitness tests ($p<0.0001$), due to a negative association between fat CSA and performance in the whole sample ($r=-0.15$ to -0.34, $p<0.0001$). Explosive power and aerobic fitness, but not core strength, were positively correlated with SSI, BSI, BMC and ToA at both sites of the tibia and radius in this group ($r=0.10$-0.33, $p<0.05$-0.0001). Correlations disappeared after adjustment for muscle CSA or lean body mass. In overweight children, explosive power and aerobic fitness showed weak associations with tibial SSI and BSI ($r=0.17$-0.19, $p<0.06$) and stronger associations with radial SSI and BSI (0.21-0.34, $p<0.05$). Explosive power and aerobic fitness, but not core strength, were negatively affected by body weight and fat mass. Overweight children also showed positive correlations between core strength and radial SSI and BSI ($r=0.25$-0.32, $p<0.005$), which remained significant after adjustment for forearm muscle CSA or lean body mass. A similar association was found between aerobic fitness and radial BSI.

Conclusion. Physical fitness is associated with strong bones in prepubertal children. This relationship is largely mediated by muscle mass. Higher fitness levels seem beneficial for forearm bone strength, particularly in overweight children, which has important implications for the prevention of forearm fractures in this population.

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CHANGES OF MUSCLE AND BONE MASS AND BONE MARKER DURING SIMULATED WEIGHTLESSNESS IN EXERCISE AND CONTROL GROUP - RESULTS FROM BERLIN BEDREST STUDY

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Objectives: In preparation of the human mars mission of the European Space Agency (ESA) countermeasures have been developed to prevent muscle and bone loss in microgravity. Assessment of the chronology of the changes of muscle and bone mass in association with bone formation and resorption markers.

Subjects and Methods: 20 male volunteers participated in a 56 days bed rest study. 10 control subjects and 10 subjects in the exercise group. Exercise was performed with resistive vibration exercise (RVE) every day with 'Galileo Space' device (NovotecMedical, Pforzheim, Germany). Blood samples have been taken weekly. Measurement of bone formation marker P1NP and bone resorption marker CTX in serum. Muscle volume/mass was measured with magnetic resonance and whole body DXA, respectively. Bone density was measured with pQCT (XCT2000, Stratec, Pforzheim, Germany) in the distal tibia.

Results: In comparison to baseline a significant loss of trabecular bone mass in the distal tibia of -3,7% ($p<0.001$) in the control group and -0,6% (n.s.) in the exercise group, respectively. Loss of muscle volume in the lower leg of -16% ($p<0.0001$) in the control group and -2% (n.s.) in the exercise group. P1NP serum level was significantly increasing in the exercise group by 41% ($p<0.0001$) and decreasing in the control group by -11% (n.s.). CTX serum level increases by 44% ($p<0.0001$) and 35% ($p<0.0001$) in the control group and exercise group, respectively.

Conclusion: Changes of bone and muscle mass and bone markers are highly related. The adaptation process of bone can be followed extremely well according to the loss of muscle mass/volume. Resistive vibration exercise enables bone formation.

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BONE EXERCISE MEASUREMENTS IN JAPANESE WOMEN: SENDAI BONE HEALTH CONCEPT STUDY

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Progressive high-impact exercise has been shown to be beneficial for bone. Previously, we presented an accelerometer-based method for recording the intensity of impact exercise in Finnish premenopausal women (1,2). Here we evaluated the applicability of the method in Japanese women.

We performed a 12-month randomized, controlled office-based exercise trial in 91 healthy premenopausal women aged from 25 to 50 years. Participants were randomly allocated to a low-impact exercise (LIE) group (46 women, exercise program tai chi and stretching exercise, 15 min 3 to 5 times per week) or a high-impact exercise (HIE) group (45

women, exercise program 5x10 (max) jumps progressively inserted during LIE, 15 min 3 to 5 sessions per week). Daily physical activity was measured with an accelerometer-based bone exercise recorder (Newtest Ltd., Finland) for one-week periods every three months. The recorder was worn on a belt close to the iliac crest. Average daily distribution of impacts at different acceleration levels (from 0.3g to 9.2g, 0g corresponding to standing) was analyzed. Office-based impact exercise had positive effects on BMD. The HIE group demonstrated a significant positive change in femoral neck BMD compared with the LIE group (0.6% vs. -1.0%, $p<0.05$). The accelerometer-based method was able to discriminate different exercise intensities. During the first six months (measurement periods I-II) there was no difference between the groups. During the last six months (measurement periods III-IV) the number of impacts at high acceleration levels (>3.3g) was more than twofold in the HIE group compared to LIE group. Exercise that included less than 100 daily high intensity impacts improved femoral neck BMD, which is similar to our previous trial in Finnish women (2).

In conclusion, the accelerometer-based method was able to discriminate different exercise intensities. By using this method we were able to follow the progression of the HIE program. Quantitative measurement of exercise intensity is valuable in ensuring the intensity and progression of the exercise program.

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PERSISTENT ACTIVATION OF SIGNALING AND GENE EXPRESSION IN OSTEOBLASTS BY FULL LENGTH PTHRP.

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Parathyroid hormone (PTH) and PTH-related protein (PTHrP) have structural similarity within the N-terminal region that allows them to act on the same G-protein coupled receptor, the PTH1R, to activate adenylyl cyclase. PTHrP contains a number of other domains of biological activity and acts as a paracrine factor in several tissues, including bone. PTHrP and PTH1R are expressed in differentiating osteoblasts, with studies in genetically manipulated mice showing that osteoblast-derived PTHrP is a key local regulator of bone remodeling. Since what is presented to the target cell physiologically in bone is more likely to be structurally similar to PTHrP than to the N-terminal regions of PTH or PTHrP, we sought to identify differences between PTHrP(1-141) and PTH/PTHrP(1-34) actions upon osteoblastic cells.

UMR 106 osteogenic sarcoma cells were treated for 12 minutes with either PTH(1-34) or PTHrP(1-141), washed extensively, and cultured for a further 30, 60, 90 and 120 minutes. At each of these times the phosphodiesterase inhibitor, IBMX (1mM), was added and cAMP assayed after 1 2 minutes. In cells treated with PTHrP(1-141) there was an increase in cAMP at all the later time points. This effect was dose-dependent, did not occur with PTH(1-34), PTHrP (1-34) or (1-84), but did so to a much lesser extent with PTHrP(1-108). In studying later cellular events, whereas full activation of Cre-luciferase by PTH or PTHrP(1-34) required treatment of cells for 4 hours, the same activation was achieved with 15 mins' exposure to PTHrP(1-141). Furthermore whereas full activation by PTH(1-34) of expression of mRNA for *c-fos* required 1 hr exposure, *ephrin B2* and *IL-6* 4 hrs, and *osteocalcin* 8 hrs, in each case full activation of gene expression at those times was achieved with 15 mins' treatment with PTHrP(1-141). The data point to distinct differences in the mechanisms of interaction of PTHrP(1-141) and amino-terminal forms of PTH/PTHrP with receptor. Together with the susceptibility of PTHrP to proteolytic degradation, this could facilitate its function as a paracrine regulator of bone remodeling.

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AMS FOR 41CA TO MONITOR EXOGENOUS CALCIUM UPTAKE IN SIMULATED MICROGRAVITY RATS

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BACKGROUND and PURPOSE: The lack of bone calcium occurs as a consequence of exposure to microgravity. The aim of this study was to demonstrate the role of a Chinese herbal prescription on bone calcium uptake in simulated microgravity rats.

METHODS: 8-week-old male Wistar rats were randomly subdivided into 3 groups: control (CON), hindlimb-unloaded (HU), and HU + Chinese medicine (HUC). Each group experienced 4 weeks, within which HU and HUC groups were hindlimb unloaded for the last 3 weeks. During the whole experiment, all rats were given 20 mg calcium (from oyster shell) daily. CON and HU groups were free to animal food and water, while the HUC group, at the same time, was given a Chinese herbal prescription. At the 11th day of hindlimb unloading, all rats were given 6.462×10^{-5} mg ^{41}Ca in prepared solution. Feces were then collected for three days after being labelled with ^{41}Ca. All animals were sacrificed at the end of 4th week and the right femur of each rat was taken out. Calcium was isolated with 3 precipitation steps and a cation-exchange column. ^{41}Ca/Ca ratio in fecese and right femur were then measured by accelerator mass spectrometry (AMS). Total Ca content in feces and the right femur was measured by inductively coupled plasma - atomic electron spectroscopy (ICP-AES).

RESULTS: The excretion of ^{41}Ca increased and absorption ratio of ^{41}Ca decreased significantly in HU compared with CON, and absorption ratio decreased by 32.7%. The absorption ration of ^{41}Ca decreased by 12.1% in HUC and had no notable difference in comparison with CON and HU ($P>0.05$). Both of the intake and uptake rate of ^{41}Ca of the rat's femur in HU notably decreased in comparison with CON and HUC ($P<0.01$, $P<0.01$), and there was no significant difference between CON and HUC ($P>0.05$).

CONCLUSIONS: Accompanied with the loss of bone calcium induced by weightlessness, the absorption ratio of ^{41}Ca and the ability of femur calcium uptake decrease as well. However, the Chinese prescription in this trial, to some extent, increases the absorption of exogenous calcium and promotes the deposition of exogenous calcium in femur.

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STUDY ON CALCIUM NET ABSORPTIVITY BY ^{41}CA LABELING CALCIUM POOL OF RATS

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^{41}Ca ($T_{1/2}=1.04\times10^{5}$ yr, pure electron capture with emission of Auger electrons and X-rays) is an ideal tracer for understanding Ca absorption, distribution, metabolism, and for assessing Ca supplements and OP treatment medicine . ^{41}Ca tracing in human body have the advantages that the long-term (years) bone resorption can be obtained, the radioactivity can be neglected (less than environmental background) and the Ca from outside of body can be identified. Accelerator Mass Spectrometry (AMS) is the most sensitive device to measure the abundance of ^{41}Ca, the sensitivity of 10^{-15} (^{41}Ca/^{40}Ca) was obtained in the China Institute of Atomic Energy.

In this experimental study, thirty 3-month-old female Wistar rats were randomly divided into two groups: normal control group and osteoporotic group. Rats in osteoporotic group were performed ovariectomy operation. Ninety days later, each rat in osteoporotic group received an intramuscular injection of 250 μ l $CaCl_2$ Solution (containing 1.4 mg Ca and 5nCi ^{41}Ca) and then received an unlabeled oral dose of 230mg calcium carbonate (containing 70 mg Ca) daily for 30 days. Sequential urine and fecal samples were collected and were analyzed for total calcium and ^{41}Ca content. Net absorptivity was obtained by the difference between oral intake of calcium and the daily fecal output (excluding endogenous calcium). The total calcium in biological samples were measured by flame atomic absorption spectrometry (FAAS) after Microwave-Digestion, Endogenous fecal calcium was calculated from the value of ^{41}Ca/^{40}Ca in fecal measured by AMS. In the measurement of ^{41}Ca, the biological samples were prepared as CaF_2 and were pressed into Al-target holders. $^{40}CaF^{-}$ ions from the negative ion source were injected into the accelerator with terminal voltage of 8.3 MV. Ca^{8+} ions were selected by an analyzing magnet and finally identified by a multi-anode detector.

The results showed that apparent absorptivity of calcium supplements were 59.15%, the net absorptivity was 69.18% and endogenous calcium of total fecal calcium was 24.56% in the osteoporotic rats. Using ^{41}Ca labeled calcium pool in vivo could obtain the net calcium absorptivity without extrinsic labeling. Therefore, the assessment method was not affected by the chemical structure and sorts of calcium supplements, and might be used in evaluating absorptivity of marketed calcium supplements.

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THE METABOLISM OF 25(OH)-VITAMIN D_3 BY OSTEOCLAST PRECURSORS REGULATES THE DIFFERENTIATION AND FUNCTION OF OSTEOCLASTS

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The primary function of vitamin D_3 has been thought to facilitate the processes necessary to maintain a healthy skeleton by regulating calcium and phosphate homeostasis. Current studies indicate that vitamin D also plays a direct role in regulating the activity of bone cells during normal bone metabolism. That is to say, 1α-dihydroxyvitamin D_3 (1,25D) maintains normocalcaemia by directly stimulating bone resorption and increasing the rate and extent of osteoblast-mediated osteoclastogenesis.

We have recently showed that the pro-hormone and immediate metabolic precursor to 1,25D, 25-hydroxyvitamin D_3 (25D), rather than 1,25D, directly affects bone mineralization in an animal model [1] . Moreover, we have demonstrated that osteoblasts are a source of extra-renal synthesis of vitamin D metabolism and convert 25D into functional 1,25D by virtue of their expression of CYP27B1 [2] . The intriguing possibility exists that osteoclasts also participate in local production of, as well as response to, 1,25D.

In this study, we have found that RAW 264.7 cell and normal human peripheral blood mononuclear cell (PBMC) expression of CYP27B1 mRNA increases during RANKL-mediated osteoclastogenesis. These results indicate the possibility that vitamin D metabolism plays an autocrine role in the osteoclast. We examined further the role of vitamin D metabolism during osteoclastogenesis, using defined serum free media (SDM) or serum devoid of existing vitamin D metabolites due to charcoal stripping. Our results indicate that the metabolism of 25D into 1,25D by osteoclast precursors results in early elevation of TRAP mRNA levels and the numbers of TRAP and calcitonin receptor (CTR)-positive multinucleated osteoclasts. Our results also indicate that vitamin D controls the resorptive activity of these cells such that in the absence of vitamin D, resorptive activity is increased. We conclude that the metabolism of vitamin D during osteoclastogenesis is important for controlling both osteoclast formation and their resulting resorptive activity. Our findings extend the notion that the skeleton is an intracrine organ of vitamin D metabolism[3].

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MAINTAINING ADEQUATE SERUM VITAMIN D LEVELS IS ESSENTIAL TO OPTIMISE BONE MINERAL VOLUME WHEN FED A HIGH CALCIUM DIET IN A MOUSE MODEL

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The relative importance of vitamin D stimulation of intestinal calcium absorption for the regulation of bone health and the direct actions of vitamin D on bone has proven difficult to characterise. To further understand the interaction between vitamin D and calcium on bone health, 8-week-old female C57Black6 mice were placed on chow diet or on one of four defined diets: 0.1% calcium (lowCa) or 1% calcium (highCa) containing either 1000IU/kg vitamin D (D+) or no vitamin D (D-) for 12 weeks. High resolution micro-computed tomography (micro-CT) live animal scans were performed on the tibial structure both prior to commencement of diet and 12 weeks later, at 20 weeks of age. Cross-sectional images of the proximal tibial metaphysis were reconstructed to obtain 3 dimensional values for bone mineral volume/total volume (BV/TV), trabecular thickness and trabecular number. Changes in BV/TV between 8 weeks and 20 weeks of age were calculated for each animal. At 20 weeks of age, BV/TV in the group fed highCa/D+ diet reduced by 25.5% from the level recorded at 8 weeks of age, which was comparable to the natural bone loss that occurs in the chow-fed animals (29.5%). However, in the group fed the lowCa/D- diet, bone loss was significantly greater (38.2%) when compared to both chow ($P<0.001$) and highCa/D+ ($P<0.001$) fed animals. Importantly, animals fed the highCa/D- diet, which were vitamin D-deplete (<5nmol/L), but were normocalcemic, were not able to maintain bone

mineral volume to levels of the vitamin D-replete controls (35.2% , P<0.001). This suggests that maintaining serum vitamin D levels is crucial to optimise bone mineral volume when fed a high calcium diet. The effects of vitamin D depletion in these animals may be explained by the reduced direct synthesis of vitamin D within bone cells. This model of dietary vitamin D and calcium manipulation is currently being applied in the transgenic mature osteoblast/osteocyte-specific VDR over-expression (OSVDR) mouse model to establish evidence for the direct and indirect role of vitamin D on the skeleton during vitamin D depletion.

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DIETARY CALCIUM AND OESTRADIOL PROTECT OSTEOCYTE DENSITY AND BONE STRUCTURE AGAINST THE EFFECTS OF OVARIECTOMY

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The anti-fracture efficacy of antiresorptive therapies is explained, in part, by its modest effects on bone mineral density and the suppression of bone turnover. However, the cellular mechanisms by which these therapies achieve these effects have not been clearly established. We have studied the effects of high (1.6%) dietary calcium and oestradiol treatment on bone remodelling in the ovariectomised (OVX) female rat. All rats were fed a 0.4% calcium diet and either Sham operated (SHAM) or OVX at 3 months of age. At 6 months, 42 female Sprague Dawley rats were either killed (SHAM6 and OVX6) or assigned to one of the following treatment groups until 9 months of age: SHAM vehicle (SHAM9), OVX vehicle (OVX9), oestradiol (E_2) (2.5 m g/kg BW/day), or 1.6% calcium supplementation (Ca). Femora were processed and resin embedded for measurements of static and dynamic histomorphometric variables including metaphyseal trabecular bone volume (BV/TV), trabecular number (Tb.N), and trabecular thickness (Tb.Th), bone formation rate (BFR), formation period (FP), osteocyte density (Ocy/BMA), activation frequency (AcF) and osteoclast surface (Oc.S) by standard techniques. Treatment of OVX6 with E_2 yielded a BV/TV (12.9+3.6%) which was significantly higher than the OVX9 (5.4 ± 1.5%) and equivalent to SHAM9 (14.8 ± 1.6%). Ca supplemented group also yielded positive effects on BV/TV (14.9 ± 2.9%, P<0.05). Both treatments reduced AcF significantly although to different extents (Ca: 2.3+0.5/yr; $E_{2:}$:0.5 ± 0.1/yr, P<0.05) when compared with OVX9 levels (3.8 ± 0.9/yr). Both treatments increased FP, although once again to markedly different extents (Ca: 9.7+0.3 days; E_2: 20.7+0.5 days, P<0.05) when compared with OXV9 levels (8.0 ± 0.2 days). Ocy/BMA was reduced in OVX9 ani mals when compared with SHAM9 (520 ± 65/mm^2 vs 784 ± 82/mm^2, P<0.05) and both Ca and E_2 treatments improved Osteocyte density (Ca: 985 ± 66/mm^2; E_2 875 ± 100/mm^2) which were at least equivalent to SHAM9 levels (P<0.05). AcF was negatively related to Ocy/BMA (R^2=0.32, P<0.005). Both E_2 and dietary calcium supplements reduce bone turnover and improve trabecular bone volume although they exhibited different levels of suppression of AcF. It is evident that increased AcF is strongly associated with a decrease in bone volume and osteocyte density suggesting a compromise in bone quality in addition to bone loss.

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ASSOCIATION BETWEEN ENDOCANNABINOID-RECEPTOR POLYMORPHISMS AND PEAK BONE MASS - RESULTS FROM THE ODENSE ANDROGEN STUDY.

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Introduction: Peak bone mass (PBM) is largely determined by genetic factors. The endocannabinoid system has been demonstrated in the skeleton, and may affect bone structure and metabolism. It is, however, unknown whether polymorphisms in the cannabinoid receptor-1 (CNR1) affect PBM in men.

Methods: Odense Androgen Study is a population-based, cross-sectional study on endocrine functions in young men (age: 20-30 years). A total of 783 men were examined; however, 83 were excluded due to either treatment with medications affecting bone, chronic diseases, or abuse of alcohol. DXA-scans (lumbar spine, total hip, femoral neck and whole-body) were performed on a Hologic 4500 densitometer. Polymorphisms were evaluated using TaqMan assays. Regression analysis was used to test for an association between genotype and bone mass. Adjustment for age, tobacco use, alcohol consumption, fat and lean body mass was performed.

Results: Of the 3 SNP's evaluated in this study, CNR8063 was found to be associated with PBM in the femoral neck (r=0.08; p=0.04) but not total hip (r=0.06; p=0.13), WB BMD (r=0.03; p=0.41) or lumbar spine BMD (r=0.01; p=0.73). CNR1048 was inversely associated with spinal BMD (r=-0.07; p=0.05), total hip BMD (r=-0.08; p=0.04), femoral neck BMD (r=-0.10; p<0.01) and WB BMD (r=-0.09; p=0.02). No significant association between CNR1422 and BMD in either region was found.

Conclusion: Common variations in the CNR1 were found to be associated with PBM in Danish men. The association was not explained by an association between the CNR1 polymorphisms and measures of obesity.

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THE ROLE OF ESTROGEN IN THE REPAIR OF OSTEOCYTES FOLLOWING PHYSICAL INJURY IN VITRO

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Bone quality is influenced by a number of factors including the accumulation of microdamage that can lead to increased risk of fracture. It has been suggested that the physical damage of osteocytes as a result of microdamage results in their cell death. Here we have used an in vitro model of osteocyte physical injury to investigate this hypothesis and to determine whether estrogen has a protective effect against cell death following osteocyte membrane disruption. Direct physical damage was applied to MLO-Y4 osteocyte like cell line using a sharp blade. Membrane impermeant dye techniques incorporating propidium iodide and Sytox green were used to determine the dynamics of osteocyte repair following injury. Generation of reactive oxygen species (ROS) was assessed using the dye 2',7'-dichlorodihydrofluorescin-diacetate (H2DCF-DA). Prior to damage, experimental cell groups were incubated for 1hr with either, 17β-estradiol (10-100nM), alone or in combination with the estrogen receptor-antagonist ICI-182780 and the kinetics of repair monitored. The potential antioxidant influence of estradiol were investigated using pre-treatment of cells with either Quinestrol (10nM), an estrogen-derivative without antioxidant activity, or Vitamin E (10nM), a potent antioxidant prior to physical damage. Direct physical injury induced significant membrane-disruption to osteocytes as evidenced by dye uptake (within < 30 seconds, 46.74%±1.18 cells versus pre-cut 9.84%±1.18 cells). Cell membrane repair occurred over a period of 30 seconds to 5 minutes. The addition of 17β-estradiol (10-100nM) caused a dose-dependent and estrogen receptor (ICI-182780)-independent increase in the rapidity of repair. ROS generation was shown to occur following physical damage. Significantly, while Vitamin E was associated with a more rapid membrane repair of a similar magnitude to 17β-estradiol the non-antioxidant moiety containing Quinestrol did not. MLO-Y4 osteocytes are able to rapidly repair following membrane-disruption. 17β-estradiol increases repair rate via an estrogen receptor-independent mechanism, that might be associated with the presence of the known antioxidant hydroxyl group in the C3 position of the steroid A ring. These data might have implications in postmenopausal osteoporosis, where estrogen withdrawal in vivo may contribute to a reduced ability of cells to repair after microdamage.

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OESTRADIOL PROTECTS 1,25 DIHYDROXYVITAMIN D FROM CATABOLISM BY CYP24 IN INTESTINAL CELLS.

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Oestrogen replacement corrects the intestinal calcium malabsorption associated with menopause or oestrogen deficiency without altering circulating 1, 25 -dihyroxyvitamin D (1,25D). We have previously shown that oestradiol inhibits vitamin D-induction of its catabolic enzyme, CYP24 in osteoblasts, but this action has not been investigated in intestinal cells. In the present study we used the enterocyte-like cell line, Caco2, to investigate the effect of 17 beta-oestradiol (E_2) on 1,25D catabolism. Cells were transfected with the -298bp CYP24 promoter-luciferase reporter construct and treated with 1,25D (10^{-8}M) ± E_2 (10^{-8}M) for 16h. CYP24 induction was assessed by a luciferase reporter assay and cell lysate 1,25D levels were determined by radioimmunoassay (IDS). Treatment with 1,25D induced the CYP24 construct 16.1(± 1.8) fold compared to ethanol vehicle (p<0.001). E_2 alone had no effect on luciferase levels, but when the 2 hormones were used in combination E_2 suppressed 1,25D induction of the construct to 11.8(± 1.4) fold (p<0.05, compared to 1,25D). At 16h the mean 1,25D level for cells treated with 1,25D alone was 1262(±184)pmol/L

and for cells treated with both 1,25D and E_2 was 1860(±115)pmol/L ($P<0.05$, post hoc). Cells treated with either ethanol vehicle or E_2 alone had 1,25D levels <10pmol/L. In summary, E_2 suppressed 1,25D induction of the -298bp CYP24 promoter-luciferase reporter construct by 27%. The remaining levels of 1,25D after 16h treatment were almost 50% higher in cells treated with both E_2 and 1,25D compared to cells treated with 1,25D alone. We conclude that E_2 inhibits maximal 1,25D-induction of the CYP24 enzyme, partially protecting 1,25D from intracellular catabolism in intestinal cells. This suggests that oestrogen replacement may improve intestinal Ca absorption by maintaining higher cellular levels of 1,25D.

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LOSS OF FUNCTION OF THE NON-GENOMIC ESTROGEN RECEPTOR GPR30 CAUSES INCREASED BODY SIZE, PERCENT BODY FAT, AND BONE MASS IN ADULT MALE MICE

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Estrogen regulation of the male skeleton was first clearly demonstrated about a decade ago with the discovery of patients deficient in aromatase or with a mutation in the ERα gene. Estrogen action on the skeleton is thought mainly to occur through the action of the nuclear receptors ERα and ERβ, with ERα apparently being more important in males. Recently, in vitro studies have suggested that the G-protein coupled receptor GPR30 is a functional ER, however its in vivo effect on the male skeleton remains unknown. We have characterized bone mass and body composition in male 4 month old GPR30 knockout mice (GPR30KO) and their wildtype (WT) littermates (n=9 per genotype) by DEXA. All mice were of a mixed C57Bl6 and 129 background. GPR30KO mice weighed significantly more ($p<0.01$) and had longer nasal anal length ($p<0.01$). Femur length and dorsal-ventral femoral mid-shaft diameter was significantly greater in GPR30KO mice ($p<0.01$ for both). By DEXA analysis, GPR30KO mice had significantly greater %body fat and whole body areal bone mineral density ($p<0.01$ for both). Additionally, femoral and lumbar spine BMD were significantly greater in the knockout mice ($p<0.05$ for both). The GH/IGF-I axis is a major determinant of body weight and skeletal growth. Serum IGF-I levels, measured by RIA after acid-ethanol extraction, were not different between genotypes. These data suggest, that in male mice, GPR30 regulates bone mass, bone size, and body composition independent of the nuclear estrogen receptors and in a manner not requiring changes in circulating IGF-I. MicroCT analysis of the trabecular compartment is underway.

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THE TRABECULAR BONE ARCHITECTURE IN PROXIMAL FEMORA OF PRIMATES WITH DIFFERENT LOCOMOTOR PREFERENCES INDICATES DIFFERENT ADAPTATION MECHANISMS

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The adaptation of the architecture of trabecular bone to habitual loads, although suggested by Julius Wolff and others more than a century ago, still defies a conclusive formulation. New approaches are opened by high resolution computed tomography (HrCT), which provides three-dimensional images of the detailed architecture of trabecular bone.

Using HrCT and advanced image analysis techniques we analyze position resolved the architecture in proximal femora of primates with different locomotor behaviors. According to their locomotor preferences the analyzed primates species are assigned to the following locomotor groups: quadrupedal walker (Papio hamadryas), springer (Semnopithecus entellus), brachiator (Symphalangus syndactylus), and climber (Alouatta seniculus). For the analysis the proximal femur was split into four regions: femoral head, femoral neck, greater trochanter, and femoral shaft. A cubic volume of interest (VOI) of size $(5mm)^3$ was moved through the proximal femur and analysis was performed on 209 positions on average. In the VOI standard morphometric paramters like BV/TV, Tb.Th and Tb.N were calculated. In addition, the local anisotropy was determined calculating the degree of anisotropy and the main orientation of the trabeculae based on an analysis of the mean intercept length.

The analysis of the relation between BV/TV, Tb.N and Tb.Th under inclusion of data from all monkeys and all different regions in the proximal femora suggest two different mechanisms of trabecular bone adaptation depending on the external loading. In highly loaded regions of the proximal femur, BV/TV increases by increasing the thickness of the trabeculae, while Tb.N remains constant. In lower loaded regions, BV/TV decreases by reducing the number of the trabeculae while Tb.Th does not change. This reduction in Tb.N goes along with an increase in the degree of anisotropy, indicating an adaptative selection of trabeculae. The main orientation of the trabeculae in the femoral head is directed towards the femoral neck. Only the gibbon, which uses mainly its arms for locomotion, displays a significantly lower trabecular anisotropy and a more radially arrangement within the femoral head.

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THE PROINFLAMMATORY CYTOKINES TNF-RELATED WEAK INDUCER OF APOPTOSIS (TWEAK) AND TNFA INDUCE THE MITOGEN ACTIVATED PROTEIN KINASE (MAPK)-DEPENDENT EXPRESSION OF SCLEROSTIN IN HUMAN OSTEOBLASTS

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We have recently shown that TWEAK is a mediator of inflammatory bone remodelling[(1)]. The aim of this study was to investigate the role of TWEAK in modulating human osteoblast activity, and how TWEAK and TNF a might interact in this context. Recombinant TWEAK and TNF were both mitogenic for human primary osteoblasts (NHBC). TWEAK dose- and time-dependently regulated the expression of the osteoblast transcription factors RUNX2 and osterix. TWEAK inhibited *in vitro* mineralization and down-regulated the expression of osteogenesis associated genes. Significantly, TWEAK and TWEAK/TNF induced the expression of the osteoblast differentiation inhibitor and *SOST* gene product, sclerostin. Sclerostin induction was mitogen activated protein kinase (MAPK) dependent. The SOST mRNA levels induced by TWEAK were on average 27.7 ± 4.7-fold within 72 hours of exposure. These levels were equivalent to or exceeded those seen in steady-state human bone and the TWEAK/TNF induction of SOST mRNA was recapitulated in freshly isolated bone fragments *ex vivo*. TWEAK induced sclerostin expression was observed in immature osteoblastic cells, both in cycling ($Ki67^+$) primary NHBC and in the cell lines MC3T3-E1 and MG63, as well as in human osteocyte-like cells and in the osteocyte cell line, MLO-Y4. Together, our findings suggest that TWEAK, alone and with TNF, is a key regulator of osteoblast function, both by regulation of transcription factor expression and by inducing sclerostin expression in both immature and mature cells. Our results also suggest new roles and modes of action for the demonstrated key regulator of bone mass, sclerostin.

(1) Perper SJ et al 2006 TWEAK is a novel arthritogenic mediator. J Immunol 177:2610-2620.

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IN VITRO AND IN VIVO EFFECTS OF ADIPONECTIN ON BONE

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Fat mass impacts on both bone turnover and bone density, and is a critical risk factor for osteoporotic fractures. Adipocyte-derived hormones may contribute to this relationship, and adiponectin is the principal circulating adipokine. However, its effects on bone remain unclear. We have, therefore, investigated the direct effects of adiponectin on bone cells *in vitro,* and determined the bone phenotype of adiponectin-deficient mice.

Adiponectin was dose-dependently mitogenic to primary osteoblasts (60% increase at 10 μg/mL), and markedly inhibited osteoclastogenesis (by 26% and 54% at 1 and 5 μg/mL, respectively). It had no effect on bone resorption in isolated mature osteoclast assays.

In adiponectin-knockout (AdKO) male C57BL/6J mice, trabecular bone volume and trabecular number (assessed by micro-computed tomography) were increased at 14 weeks of age, by 30% (p=0.02) and 38% (p=0.0009), respectively. Similar, non-significant trends were observed at 8 and 22 weeks of age. Biomechanical testing showed lower bone fragility and reduced cortical hardness at 14 weeks.

We conclude that adiponectin acts directly on bone cells, but that these actions do not explain the bone phenotype of the knockout animals. Thus, it must also have indirect effects on bone, possibly through modulating growth factor action, or insulin sensitivity. Since adiponectin does influence bone mass *in vivo*, it is likely to be a contributor to the fat-bone relationship.

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POSITIVE BMD RESPONSE FOLLOWING NITROGLYCERIN THERAPY IS ASSOCIATED WITH ELEVATION OF SERUM IGF-1 LEVELS

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Aim: Cost-effective therapies are necessary for prevention and treatment of osteoporosis. Use of hormone replacement therapy (HRT) has declined after Women's Health Initiative clinical trial. At appropriate doses, nitroglycerine, a nitric oxide (NO) donor has favorable effects on osteoblasts and osteoclasts. The beneficial effects of estrogen on bone are mostly mediated via IGF-1 and NO/cGMP pathways. Since therapy with IGF is not feasible, use of NO donor therapy to obtain estrogen-related beneficial effects on bone was explored.

Method: A three-year randomized, double blind, controlled clinical trial (n=186) was conducted to assess the efficacy of nitroglycerine in preventing bone loss in early postmenopausal women [Nitroglycerin as an Option: Value in Early Bone Loss (NOVEL study]. Women were randomized to receive nitroglycerine ointment or placebo ointment. There were no differences in the treatment arms in key baseline characteristics. Intent to treat analysis demonstrated no difference in lumbar spine BMD. But taking compliance (~75%) into consideration, the actual dose used by the study participants is ~50% of that was originally intended to be used.

Results: Those subjects who had increased BMD following Nitroglycerine-therapy had highly significant increase ($p<0.001$) of serum IGF-1 levels (201 ± 25.6 vs. non-responders, 40.2 ± 16.9). In comparison, those subjects who had increased BMD in the placebo-treated group had no change in serum IGF-1 levels (-2.6 ± 24.6 vs. 10.8 ± 13.5; responders vs. non-responders, respectively). Furthermore, in the nitroglycerin-treated group, the BMD changes observed were highly correlated with the change of serum IGF-1 levels from the baseline ($r = 0.5$; $p<0.01$). However, there was no correlation seen with the change of BMD with the baseline serum IGF-1 levels.

Conclusion: In this study, those who had increased BMD in response to nitroglycerin therapy had a significant increase in serum IGF-1 levels. This positive correlation is restricted to nitroglycerin-treated group only, suggesting that increase in serum IGF-1 levels may be used as a marker to identify BMD responses, dose-adequacy, and the adherence to therapy. These results open new avenue to understand the mechanism of actions of nitric oxide in bone.

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SERUM OSTEOCALCIN LEVEL IS ASSOCIATED WITH GLUCOSE METABOLISM AND ATHEROSCLEROSIS PARAMETERS IN TYPE 2 DIABETES MELLITUS

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Introduction: Recent animal studies showed that osteocalcin action is related to not only bone metabolism but also to glucose metabolism and fat mass. We investigated the relationship between two bone formation markers, serum osteocalcin and bone specific alkaline phosphatase (BAP), and glucose metabolism, serum adiponectin, and the amount of fat mass as well as atherosclerosis parameters in men and postmenopausal women with type 2 diabetes. Methods: A total of 179 men and 149 postmenopausal women were recruited consecutively, and radiographic and biochemical characteristics were collected. Brachial-ankle pulse wave velocity (baPWV) and intima-media thickness (IMT) were evaluated as the parameters of atherosclerosis. Results: Multiple regression analysis adjusted for age, duration of

diabetes, body mass index, and serum creatinine showed that osteocalcin negatively correlated with fasting plasma glucose and HbA1c in both men and postmenopausal women ($p<0.05$) and with %Fat, baPWV, and IMT in men ($p<0.05$). Osteocalcin positively correlated with total adiponectin in postmenopausal women ($p<0.001$). After additional adjustments for systolic blood pressure, LDL-cholesterol, HDL-cholesterol, HbA1c, and Brinkmann index, osteocalcin still significantly and negatively correlated with baPWV and IMT in men. In contrast, osteocalcin did not correlate with fasting C-peptide, and BAP did not correlate with any variable in either men or postmenopausal women. Conclusions: Serum osteocalcin is associated with glucose and total adiponectin levels, fat mass, and atherosclerosis parameters in patients with type 2 diabetes, suggesting that osteocalcin is important for not only bone metabolism but also glucose and fat metabolism.

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OSTEOINDUCTIVE CAPACITY AND HEAT STABILITY OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN-2 PRODUCED BY *ESCHERICHIA COLI* AND DIMERIZED BY BIOCHEMICAL PROCESSING

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One problem associated with clinical application of CHO-derived recombinant human bone morphogenetic protein (C-BMP-2) is its high cost due to the need for use of high doses. To solve this problem, E. coli-derived BMP-2 (E-BMP-2) has been examined using the technique of molecular unfolding and refolding. However, it is unclear whether the characteristics of E-BMP-2 are appropriate for clinical application. In this study, we examined the biological activity of E-BMP-2 and its heat tolerance in in vitro and in vivo systems. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) confirmed the high purity of E-BMP-2. EBMP-2-induced alkaline phosphatase (ALP) expression in osteoprogenitor cells (C2C12, ST2, and primary murine calvarial osteoblast cells) was dose-dependent, and consistently elicited ectopic new ossicles of significant size in mice, also in dose-dependent fashion. In addition, E-BMP-2 induced phosphorylation of Smad1/5/8 and mRNA expression of osteoblastic differentiation markers to the same extent as C-BMP-2. On the other hand, when E-BMP-2 was exposed to increasing heating temperatures and times, its bone-inducing capacity was maintained until heating at 70°C for 2 hours or 90°C for 15 minutes. Thus, E-BMP-2 will exhibit decrease in activity with the sterilization procedures required prior to use in surgery. These findings indicate that the biological capacity and heat stability of E-BMP-2 are almost equivalent to those of currently available C-BMP-2, and suggest that E-BMP-2 might thus solve current problems of cost impeding routine clinical use of rhBMP-2.

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ROLE OF NEUROPEPTIDE Y IN CONTROL OF BONE AND ADIPOSE HOMEOSTASIS IN ANDROGEN DEFICIENCY.

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The hypothalamus is the origin of endocrine and neural pathways that regulate bone mass, suggesting the potential for interaction in their regulatory influence on bone. We have recently shown an interaction between androgens and neuropeptide Y (NPY), a critical component of hypothalamic signalling, in control of osteoblast and adipocyte activity. The anabolic phenotype of NPY Y1 receptor deficient mice ($Y1^{-/-}$) was completely attenuated by orchidectomy (ORX), however, this attenuation was not evident following ORX in $Y2^{-/-}$ mice . This differential response between $Y1^{-/-}$ and $Y2^{-/-}$ mice suggests a receptor-specific interaction between androgens and NPY.

In order to further investigate this interaction, the skeletal response to ORX was examined in NPY deficient mice in comparison with $Y1^{-/-}$ and $Y2^{-/-}$ mice. Mice underwent ORX or sham-operation at 8 weeks of age and skeletal responses were examined at 16 weeks of age.

White adipose tissue (WAT, g) was decreased post-ORX in wild type (0.98 ±0.08 vs 0.58 ±0.19, $p<0.05$). This loss was absent in $NPY^{-/-}$ (0.73 ±0.05 vs 0.72 ±0.08), this is similar to $Y1^{-/-}$ which gained WAT post ORX (0.81 ±0.02 vs 1.28 ±0.14, $p<0.01$) . ORX reduced cancellous bone volume (BV/TV, %) in wild type (7.7 ±0.9 vs 3.5 ±0.2, $p<0.0001$) and $NPY^{-/-}$ (13.4 ±1.8 vs 6.5 ±0.5, $p=0.001$) however, despite this BV/TV in ORX-$NPY^{-/-}$ mice remained greater than ORX-wt ($p<0000.1$). This osteopenia was the result of reduced trabecular number in wt (data) and $NPY^{-/-}$ (3.7/mm ±0.2 vs 2.0 ±0.1, $p<0.0001$). The NPY-mediated anabolic phenotype involves greater mineral apposition rate (MAR, μm/d).

MAR in wild type mice was not affected by ORX (data) but was increased in *NPY*$^{-/-}$ (1.7 ±0.06 vs 2.0 ±0.12, p=0.02). This is in contrast to the loss of MAR post-ORX in *Y1*$^{-/-}$ compared to ORX-wild type mice (wt 1.26 ±0.6 vs *Y1*$^{-/-}$ 1.34 ±0.1).

Taken together, these studies indicate specific interactions between NPY and androgen signalling in the control of fat and bone tissue. For fat tissue, Y2 signalling appears to regulate the loss of fat post-ORX, while in bone Y1 signalling appears to maintain osteoblast activity post-ORX.

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N-3 AND N-6 LONG-CHAIN POLYUNSATURATED FATTY ACIDS DIFFERENTIALLY MODULATE IL-6 SECRETION IN LIPOPOLYSACCHARIDE-STIMULATED MURINE OSTEOBLASTS

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Pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α are known to be active in the pathogenesis of osteoporosis. Results from clinical trials and in vivo animal studies suggest that specific long chain polyunsaturated fatty acids (LC-PUFAs) especially those of the n-3 PUFA family, might be beneficial for bone health. In order to elucidate possible cellular mechanisms, the effects of some LC-PUFAs, representative of the n-3 and n-6 families, were investigated on osteoblastic secretion of various inflammatory cytokines.

Lipopolysaccharide-stimulated murine MC3T3-E1 osteoblasts were exposed to ethanol (vehicle control), arachidonic acid (AA), gamma-linolenic acid (GLA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 20 μg/ml for periods of 2.5h to 20h. Conditioned media were collected and cytokine levels determined with a Mouse Th1/Th2 10plex FlowCytomix Multiplex kit (BenderMed Systems) and expressed in pg/ml. Three independent experiments were conducted (n=4).

Negligible levels of the pro-inflammatory cytokines IL1-α and TNF-α were detected in the conditioned media of all samples tested. Compared to the respective controls, AA (n-6) stimulated IL-6 secretion significantly by 140-200%, which was evident after 2.5h already. GLA (n-6) and EPA (n-3) enhanced IL-6 secretion by 10-80%, reaching a maximum after 10-15h of exposure. DHA (n-3) had a pronounced inhibitory effect on IL-6 levels.

Prostaglandin E2 (PGE2) has been shown to stimulate IL-6 secretion. The stimulatory effect of AA on IL-6 secretion could be attributed to the production of PGE2 that is derived from AA. In our study, this is confirmed by the observation that co-incubation of AA with the cyclo-oxygenase-2 blocker NS-398 (20 μM) significantly attenuated stimulation of IL-6 secretion. Enhancement of IL-6 secretion by GLA and EPA may be associated with production of PGE1 and PGE3 respectively. No prostanoids are derived from DHA, suggesting that the inhibitory effect of this n-3 LC-PUFA on IL-6 secretion could act through a different pathway. Further work is needed to understand the modulation of various cytokines by the LC-PUFAs in bone cells.

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SERUM VITAMIN D LEVEL AND BONE MINERAL DENSITY IN PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN IN CROATIA

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Aim: To evaluate the vitamin D status and bone mineral density (BMD) of premenopausal and postmenopausal women in Croatia and to assess the probable correlation of vitamin D serum level with BMD.

Material and methods: We conducted a cross-sectional observational study of 790 women. The age range was 44-78 years. Serum 25(OH)vitamin D was assayed in each patient. Spine and proximal femur BMD were measured by dual energy X-ray absorptiometry (DEXA). Women were divided into subgroups. The first group consisted of 70 younger women with regular menstrual status, second group was consisted of 240 postmenopausal women within 5 years since menopausa, and the third group was consisted of 480 postmenopausal women with more than 5 years since menopausa.

Results: Based on data in the literature, we used four 25(OH)D cutoffs to define vitamin D deficiency: 30, 50, 75, and more than 75 nmol/L. Vitamin D serum level was found in 8.02%, 24.1%, 30.3%, and 36.7% with 30, 50, 75, and more than 75 nmol/L cutoffs, respectively. The mean serum 25(OH)D level was 60.81+/-26.1 nmol/L, 67.25 +/- 21.4 nmol/L, and 68.86 +/- 18.1 nmol/l with the first to the third group of women, respectively. 25(OH)D serum level slightly increases with age in Croatian women but not statistically significant. Measurement of BMD by DEXA have

shown that osteopenia or osteoporosis are even present in younger women with regular menstrual status. Such women have lower than 30 nmol/L of serum vitamin D level. The number of women who has osteopenia or osteoporosis is increased with age. In these groups serum vitamin D level is in negative correlation with BMD. In the third group the prevalence of osteoporosis is the highest. The mean characteristics of the first group of women is that deficiency of vitamin D correlates with osteopenia or osteoporosis.

Conclusion: Vitamin D deficiency is common among premenopausal and postmenopausal women with osteopenia or osteoporosis in Croatia (T-score less than or equal to -2.5). We found correlation among low serum vitamin D level and osteopenia or osteoporosis in younger women with regular menstrual status.

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THE FORKHEAD TRANSCRIPTION FACTOR FOXC2 STIMULATES OSTEOBLAST DIFFERENTIATION

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The forkhead box C2 (Foxc2) protein is a member of the family of winged helix/forkhead transcription factors. Foxc2-deficient mice display defective formation of the aortic arches, multiple craniofacial bones, and vertebral column. However, little is known about how this transcriptional factor functions in osteoblast differentiation. To investigate the role of Foxc2 in osteoblast differentiation, DNA containing Foxc2 was transfected into the developing cranial suture mesenchymal cells by electroporation. Compared to the control, alkaline phosphatase (ALP) and bone sialoprotein (BSP) were expressed strongly in suture mesenchymal cells in the Foxc2 overexpressed calvaria. After Foxc2-siRNA transfection, ALP staining was rarely observed in the suture mesenchyme and adjacent parietal bone of the calvaria. Furthermore, Foxc2-siRNA treated cells showed decreased ALP and Alizarin red staining compared to the control during induction of osteoblast differentiation in MC3T3-E1 cells. Foxc2 overexpression increased active b-catenin levels and stimulated T cell factor/lymphoid enhancer factor (TCF/LEF) transcriptional activity in both MC3T3-E1 preosteoblasts and C3H10T1/2 cells. The protein kinase A (PKA) inhibitor H-89 suppressed Foxc2-mediated increase of TCF/LEF transcriptional activity in C3H10T1/2 cells (~ 40%, P <0.01). In addition, Foxc2 enhanced Runx2 transcriptional activity without changing Runx2 expression in the preosteoblasts. In conclusion, our results demonstrated that Foxc2 stimulated osteoblast differentiation of mesenchymal cells and preosteoblasts. Activation of canonical Wnt- b-catenin signals might be involved in the Foxc2-mediated stimulation of osteoblast differentiation. At the least, activation of Runx2 plays a role in the stimulation of differentiation by Foxc2 in preosteoblasts.

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POSSIBLE ROLES AND POTENTIAL APPLICATIONS OF THE $P2X_7$ RECEPTOR IN HUMAN BONE CELLS

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Effective bone remodelling is a coordinated process involving a balance between bone formation and bone resorption, carried out by osteoblasts and osteoclasts respectively. Recently, the P2X7 receptor has been proposed as a component of a potential bone regulatory system. This plasma membrane receptor is an ATP-gated ion channel, activated by an ATP analog, BzATP. In a recent report, two types of inactivating P2X7 receptor polymorphisms were associated with an increased fracture risk in postmenopausal women (1). In preliminary studies, patients with P2X7 receptor mutations show a decrease in bone density, supporting the proposal that this receptor is involved in bone turnover (2). In the current study, it was confirmed by immunohistochemistry that the P2X7 receptor was expressed by human primary osteoblast-like cells. Using fluorescence microscopy, the functionality of the P2X7 receptor was established by a prominent increase in the uptake of ethidium bromide into osteoblast nuclei after treatment with BzATP. There was no evidence that stimulation of the receptor affected osteoblast survival, with or without the application of stress to the cells. However, osteoblast proliferation was found to be significantly increased at the lowest BzATP concentration of

0.001mM ($p<0.001$) and significantly decreased when treated with 1mM BzATP ($p<0.001$). Furthermore, treatment of human osteoclast precursors, derived from buffy coats of blood, with BzATP altered osteoclastogenesis. A 4-7 fold increase in pits on dentine were found in the groups treated with 0.01-0.1mM BzATP compared to controls ($p<0.001$). In addition, a nearly 2-fold increase in the number of multinucleated osteoclasts at late stages of culture with 1mM BzATP ($p<0.01$) indicates a potential role for the P2X7 receptor in regulating osteoclast lifespan. These assays can be extended to precursor osteoclasts derived from blood samples of patients with P2X7 receptor mutations to further our understanding of its role in bone cell function.

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IN VITRO RESPONSE OF PRIMARY HUMAN BONE MARROW STROMAL CELLS TO RHBMP-2 IN THE EARLY AND LATE STAGES OF OSTEOBLAST DIFFERENTIATION

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A number of factors must be added to human bone marrow stromal cells (hBMSCs) in vitro to induce osteogenesis, including ascordic acid, β-glycerophosphate, and dexamethasone (Dex). Bone morphogenic protein (BMP)-2 is an osteoinductive factor that can commit stromal cells to differentiate into osteoblasts . However, it is still not clear whether the addition of BMP-2 alone in vitro can induce hBMSCs to complete osteoblast differentiation, resulting in matrix mineralization. This study compares the effects of BMP-2 and Dex, alone and combined, on the early and late stages of hBMSC differentiation . We found that BMP-2 causes a significant induction of alkaline phosphatase (ALP) activity in hBMSCs, with a transcriptional up-regulation of known BMP-2-responsive genes, and the stable expression of cbfa1 in the nucleus and the regions surrounding the nucleus in the early phase of osteoblast differentiation. However, continuous treatment with BMP-2 alone at doses ranging from 100-300 ng/ml results in a less efficient enhancement of in vitro matrix mineralization, despite a significant induction of ALP activity at a concentration of 100 ng/ml. Our results reflect how the effects of BMP-2 on hBMSCs can vary depending on the stage of osteoblast differentiation, and highlight the need to understand the role of BMP-2 in primary hBMSCs derived from diverse sources in order to increase the efficiency of using BMP-2 in osteoinductive therapies.

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Category 2. Osteoporosis: Assessment and Treatment

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THE EFFECTS OF EARLY INTRAVENOUS PAMIDRONATE TREATMENT ON INFANTS WITH MODERATE TO SEVERE OSTEOGENESIS IMPERFECTA

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OBJECTIVE: To report the effects of intravenous pamidronate in children less than 12 months with moderate to severe Osteogenesis Imperfecta (OI).

PATIENTS AND METHODS: A retrospective review of 10 patients (9 females) with moderate to severe OI who started pamidronate at age less than 12 months. All received at least 12 months of treatment. Records were reviewed to determine age of diagnosis, and infusion history. Anthropometry, fracture history, bone mineral density (DXA) and mineral homeostasis were assessed prior to treatment and12 months. Ages at which major motor milestones were achieved were also determined.

RESULTS: Two patients were diagnosed antenatally, 5 at birth and one each at 3, 12 and 24 weeks. Postnatal diagnoses were made by clinical and radiological phenotype. Mean age of pamidronate start was 7.2 months (range 1.8 -11.8, SD+/- 3.4 months). Seven patients received 12mg/kg/year of pamidronate while three received 9mg/kg/year. 80% had acute phase reaction with first infusion.

Prior to start the annualised fracture rate was 9.1 with a mean number of long bone fracture of 5.5±3.8 (range 2-14) and 5 (50%) had vertebral compression fractures. Over the 12 months of treatment, the fracture rate decreased to 0.9/year, height and weight SD remained stable, lumbar spine BMD (LSBMD) increased and mineral homeostasis was not affected (Table). Vertebral shape improved in those with pre-existing collapse.

Motor milestones were achieved at the following median ages in months: rolling 10, independent sitting 14, crawling 12.5, pulling to stand 16 and walking 23.

CONCLUSION: Intravenous pamidronate started <12 months of age in children with moderate to severe OI was well tolerated and associated with an increase in LSBMD, reduced fracture frequency, normal growth and vertebral modeling. Motor milestones were achieved earlier than previously published (Engelbert, et al, 2000).

Table. Height, weight, bone density and biochemical data at baseline and 12 months. Data presented as mean (SD).

	Baseline	12 months	P value
Weight (z-score)	-3.1 (1.7)	-2.9 (1.7)	>0.5
Height (z-score)	-2.7 (1.7)	-2.4 (2.1)	>0.5
Lumbar spine BMD (z-score)	-3.3 (0.9)	-1.8 (1.8)	0.03
Calcium (mmol/L)	2.48 (0.09)	2.45 (0.08)	0.6
Alkaline phosphatase (IU)	312 (253)	220 (115)	0.2

(1) Engelbert RH, et al, Eur J Pediatr. 2000 Aug;159(8):615-20

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STATE OF THE ART OSTEOPOROSIS DIAGNOSIS

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Mineral bone density is only mineral bone density, risk factors for osteoporosis are only risk factors and mixing of both parameters does not make quite more sense, It is not best than each of them alone. Bone density is a subrogate parameter for diagnosing bone strength is good but is not enough, because with a suitable bone density caused by sodium fluoride bone fragility is increased and with decreased mineral bone density some individuals undergo bone fractures and a person other does not suffers this bone condition. And testing in cadaver is good but not enough because bones are alive structures and not only anything static but dynamic and in motion. Measurement of bone mineral density by dual energy X-ray absorptiometry (DXA) has served as a fit surrogate for the measurement of bone strength and accounts for approximately 70 percent of bone strength or less. It is necessary to be very carefull when using models for data inference, because we obviously will never know the underlying truth contained in the data. Therefore, it is tried to regain some information about the third dimension by building a model of the bone, which assumes axial symmetry. And here is the problem and the answer to this problem. Assumptions must be made regarding the tridimensional nature of the bones, dealing with an inference problem from a set of measurements.

Bone risk factors are good but not necessarily one disease and neither enough. With and without them there are persons with and without suitable bone strength and with and without fractures. It is important to understand that bone is not a hard and lifeless structure; it is, in fact, a complex, living tissue. So, risk factors for cardiovascular events are a very good example for this question. But which is the best testing for cardiovascular conditions?. There are a lot. And all are good but which is the best. Maybe the Halter monitoring?. Maybe this is the answer to. And maybe here is the answer to osteoporosis diagnosis.

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THE IMPORTANCE OF SOCIAL, REGIONAL, EDUCATIONAL AND NUTRITIONAL FACTORS IN OSTEOPOROTIC TURKISH FEMALES

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Practical treatment of patients with OP requires pharmacologic interventions, physical and rehabilitative measures, and good nutrition. In regard to osteoporosis (OP), the distinct effects of nutrition, exercise, hormones, and lifestyle on osteoporosis cannot be separated. Public education can contribute to prevention, better understanding, and management of the consequences of osteoporosis. The aim of this study was to evaluate the social, educational, and nutritional factors in 415 osteoporotic Turkish females.

The mean age of these patients was 64.8 ±10.5 years. Most of the patients had primary osteoporosis (80.8%) with nearly equal distribution (51% postmenapausal, 49% senile). Mean menapausal age was 45.5 ±6.3 years. Routine tests recommended by American Association of Clinical Endocrinologist (1) were in normal limits except mildly low 25-OH D levels (74.5 ±61.6 nmol/mL). Socio-demographic profiles were striking including low educational level (illeterate 55.6%, elementary school graduates 37.7%), high numbers of pregnancy/birth/long nursing (69.9%). Nutritional profile for diet lacking for calcium (53.9%) were prominent. Nearly all of the patients have used calcium plus vitaminD drugs (99.7%). Tobacco use and alcohol consumption were extremely low (2.2%). More strikingly, 87.2% of patients had risk of lack of sun-light (only face and hands) because of their traditional islamic clothing style. Biphosphonates have been used in the most of the patients (40.3%), calcitonin (21.1%), strontium (8.2%), calcitriol (5.3%), alfacalcidiol (3.2%), raloxifene (2.7%) and hormon replacement therapy were followed them. Pharmacologic treatments have been changed in 85.4% of patients.

The regional facts may be more important than global reality of osteoporosis. Preventive strategies should be developed according to these regional facts such as social factors including education, women-health issues, nutrition, and adequate sun-exposure.

(1) www.aace.com

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EFFECTS OF NUTRITIONAL SUPPLEMENTATION ON BONE DURING WEIGHT REDUCTION IN MICE

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Obesity is a global epidemic and many adopt weight loss strategies including food restriction (FR) and taking nutritional supplements. Although it is very well established that FR (40% less food) decreases body weight and increases lifespan in animal models, it has also been reported to induce bone resorption leading to net loss of bone. There is recent evidence that n-3 fatty acids (n-3 FA) and conjugated linoleic acid (CLA), when supplemented in the diet, attenuates age-related and postmenopausal bone loss. We hypothesize that n-3 FA can nullify the effects of FR on bone and CLA can reduce ovariectomy induced bone loss. We studied 1. Effects of FR on fat-1$^+$ mouse model (endogenously produces n-3 FA), and 2. Effects of CLA on ovariectomy induced bone loss in female middle aged C57Bl/6 mice.

Expt 1. Proximal tibial metaphysis (PTM) of fat-1$^+$ FR mice had higher cancellous bone mineral content (Cn BMC), cancellous bone mineral density (Cn BMD), cortical bone mineral content (Ct BMC), cortical bone mineral density (Ct BMD) cortical thickness (Ct Th) and periosteal perimeter (Peri PM). In the distal femoral metaphysis (DFM), there was increased Cn BMC, Cn BMD, Ct BMC, Ct BMD and Ct Th. There were no changes in the Peri PM, but fat-1$^+$ FR mice had decreased endocortical perimeter.

Expt 2. In the PTM, Cn BMC of the CLA fed sham mice increased significantly, when compared to that of CO sham mice. After OVX, CLA fed mice showed significantly higher Cn BMC and BMD. In the DFM, CLA fed mice had higher Cn BMD and Ct BMC. After ovariectomy, CLA fed mice had increased cancellous and cortical bone, but these increases were not statistically significant.

We conclude that a combination of FR and n-3 FA protected femur and tibia from bone loss. CLA also protected ovariectomy induced bone loss. During weight loss regimens, n-3 FA supplementation may prevent bone loss while CLA supplementation, while helping weight loss, will also maintain and protect bone mass.

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OESTROGEN ISSUE IS NOT SOLVED FOR OSTEOPOROSIS?.

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The 46,XX gonadal dysgenesis may be sporadic or familial is characterized by "streak gonads", is a type of female hypogonadism in which no functional ovaries are present to induce puberty. The streak gonad is incapable of ovulation or estrogen secretion. The syndrome is sometimes called "pure gonadal dysgenesis". Familial XX gonadal dysgenesis is transmitted as an autosomal recessive trait and its locus was mapped to chromosome 2. The syndrome is sometimes called “pure gonadal dysgenesis”(PGD) . Patients with PGD have a normal chromosomal constellation but may have localized genetic alterations. Because of the inability of the streak gonads to produce sex hormopnes most of the secondary sex characteristics do not become developed.

These phenotypic females are characterized by a normal stature, sexual infantilism, bilateral streak gonads, amenorrhea, elevated plasma LH and FSH concentration . We report a patient twin pair who presented at age 14 years with absence of development in feminization, with a history of primary amenorrhea. They had poorly developed breasts, a hypoplastic uterus, a normal vagina and infantile genitalia. Their karyotype in leukocytes and fibroblasts was 46,XX, and streak gonads examined by laparoscopy. Replacement treatment with oestrogen plus progestin resulted completely in feminization improvement. most of these girls will develop pubic hair, though it often remains sparse because the adrenal glands can make limited amounts of androgens.

Aged 36 years densitometry was performed and in lumbar spine osteoporosis were found with t-score -3.3, -3 and oestrogen plus progestin were good for menstrual periods but not for lumbar spine calcification. Where is the failure of oestrogen? Maybe is not good whichever oestrogen for preventing osteoporosis?.

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ALWAYS IS MILK GOOD FOR CLIMACTERIC OSTEOPOROSIS ANYWAY?

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Objective: to determine if milk is better than calcium carbonate for bone health.

Material and method: worldwide bibliography review on the problem.

Results: life is a struggle against hydrogen ions. Long-term acid loading in humans causes an increase in renal acid excretion. People taking high doses of PPIs are more likely to break a hip. PPIs interfere with the continuous breakdown and rebuilding of bone. Dietary protein has a magnitude-dependent anabolic effect on bone, because protein supplies the amino acid substrates for building bone matrix. Contemporary Western diets contain acid precursors in excess of base precursors. Consumption of animal protein, grain, and high amounts of milk increases the acidity of the body. Acidosis influences the homeostasis of calcium, partly due to the influence on renal mechanisms. Scant evidence supports nutrition guidelines focused specifically on increasing milk or other dairy product intake for promoting child and adolescent bone mineralization. In humans, essential amino acid supplementation increases circulating concentrations of insulin-like growth hormone. The catabolic effect on bone related to the magnitude of the diet's net acid load can offset the anabolic effect of higher dietary protein intakes. Sodium chloride, elevates urinary calcium excretion. Higher long-term protein intakes are associated with the bone variables as an anabolic factor, whereas higher long-term diet-dependent net acid loads are associated with those variables as a catabolic factor. The net effect proved anabolic but is apparently short of protein's anabolic potential because of the catabolic effect of the positive net acid load that causes calcium urinary loss induced by acidity. Ca from Ca-rich mineral waters is equivalent to that of milk Ca but the acidogenic action of SO4 is responsible for the increased calciuria. Consumption of fruits and vegetables has been implicated in lowering net acid excretion. The association between fruit and vegetable consumption and indexes of bone health was first identified within the older population by the alkalizing effect of fruit and vegetable consumption.

Conclusion: milk without acid/base balance diet is not a good παραδείγμα versus calcium carbonate for postmenopausal osteoporosis in calcium urinary loss.

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OSTEOPOROSIS DIFFICULT TO DEAL WITH IN HIV POSITIVE PATIENTS.

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Issues: There is a problem of osteoporosis in HIV-positive persons. Bone disorders have emerged as complication for adults and children, offsetting any quality-of-life advantages gained by current use of antiretroviral. There is conflicting evidence on which specific drug classes are more likely to. But the problem is measurement of BMD.

Description: So, to measure strength bone involve a great deal to cope with. DXA has served as a fit surrogate for. By reason of the two-dimensional nature of DXA, assumptions must be made regarding the tridimensional nature of the bones involving a great deal to cope with.

Lessons learned: Therefore it is deduced, that this method seem to be very sensitive to error, and it is necessary to know how to deal with these errors, especially with the systematic errors introduced by using a parameterized model. Significant discordance in longitudinal changes in BMD was observed.

Next steps and conclusions: So, a mathematical, physical and physiological 5-dimensional model must be developed in order to gauge bone properties including geometry (2-dimensional DXA) , space, time, motion and stress with some portable-computer-devices in base of mouse models for quantitative trait loci (QTL) analyses. In the field of skeletal micro-structure, μ CT has proven to be an invaluable imaging tool and the use of high resolution peripheral quantitative computed tomography (HR-pQCT), in vivo nanotomography and nanofractography, must be considered for studies of bone disease and its treatment, and here must stay new university research methods for new times and new pathologies.

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EFFECTS OF 8 MONTHS OF TWICE-WEEKLY HIGH VERSUS LOW INTENSITY WHOLE BODY VIBRATION ON RISK FACTORS FOR HIP FRACTURE IN POSTMENOPAUSAL WOMEN: A RANDOMIZED CONTROLLED TRIAL

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INTRODUCTION Whole body vibration (WBV) may improve bone mass and reduce fall risk in postmenopausal women at risk of hip fracture, however, the minimum effective dose and relative efficacy of high versus low intensity vibration, are unknown.

METHODS Forty-seven postmenopausal women were randomised to control, low intensity (LV) or high intensity (HV) vibration. At baseline and follow-up, biometrics, whole body, proximal femur (PF), lumbar spine (LS) and forearm bone mineral content (BMC), bone mineral density (BMD), LS area, FN area, cortical width, index of bone strength, cross sectional moment of inertia (XR36, Norland), calcaneal broadband ultrasound attenuation (BUA) (QUS-2, Quidel), and functional muscle (chair rise, wall squat) and balance (single leg stance, tandem walk) performance were tested. Dietary calcium was determined via questionnaire and FoodWorks analysis (2007, Xyris, Brisbane). Current and historical bone-relevant physical activity was determined using the BPAQ (1). Treatment groups attended supervised WBV sessions twice weekly for 8 months (LV – 1 x 15 minute session at 30Hz, 0.2g; HV – 2 x 3 minute sessions at 12.5 Hz, ~1g). Treatment effects were examined by intention to treat repeated measures ANOVA and multiple regression analyses controlling for age, weight, height, calcium consumption and physical activity.

RESULTS No significant between-group effects were found for any measure, however, trends were evident and within-group effects were observed. Significant bone loss occurred at the hip and spine in control ($p=0.02$) but not WBV groups. Wall squat time ("muscle strength/endurance") and single leg stance ("static balance") improved in the HV group ($p=0.004$) and a similar trend was evident for the LV group but not controls. A significant difference in calcaneal ultrasound between control and HV groups at baseline disappeared at follow-up as a result of bone loss in controls and gain in the HV group.

CONCLUSIONS Findings suggest that twice-weekly WBV cannot entirely prevent age-related skeletal loss, but that loss may be considerably muted. Muscle and balance function can be improved with very minimal exposure to high intensity WBV. Findings have important clinical implications for groups at high risk of hip fracture who are unable or unwilling to adopt traditional therapeutic options.

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COMPARISON OF THE NEW QUS PARAMETERS FROM THE NOVEL QUS MACHINE (LD-100) TO DXA AND FORMAL QUS PARAMETERS

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As we know, the different in weight bearing on each bone effects the different in bone density and bone composition of cortical bone and cancellous bone. Those two forms of bone grades into one another without a sharp boundary, even they are much different in mass and metabolic turnover rate. Concerning the Biot's theory, the novel QUS machine (LD-100) was established, providing 5 parameters with the new concept in detection of each bone status. After the confidential in in vitro experiment, LD-100 was developed for clinical practice to this study. One hundred and nine Japanese patients (62 female and 47 male) aged 49 +/- 17 years were assessed at left forearm with LD-100 (QUS), at lumbar spine and at left forearm with Lunar (DXA), and at left calcaneous with Achilles+ (QUS). We found that the pearson's correlation of BMD or bone mineral density (DXA at spine) to BUA or broadband ultrasound attenuation and BV/TV or cancellous bone volume fraction (LD-100) were moderate (0.48 and 0.46 respectively), while to BMD (DXA at forearm) and BUA (Achilles+) were also moderate (0.47 and 0.45 respectively). The most attractive for all crossover comparisons were the good correlation of the same site by different technology as at left forearm by LD-100 (BUA) and DXA (BMD) was 0.69 and the same technology on the different site as LD-100 (BUA) at forearm and Achilles+ (BUA) at calcaneous was 0.64. Also the radius thickness (Rd.Th) parameter showed increasing correspondant with the patients' age increasing. Conclusion : Lunar, LD-100, and Achilles+ can interpret the overall bone density, moreover, LD-100 can interpret more bone status as BV/TV, Rd.Th, Ct.Th, and Cn.El. Those informations will clarify not only for diagnostic of osteoporosis but also for future advance research in drug formation and its effective dose studying.

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AGE-RELATED DIFFERENCES IN THORACIC AND LUMBAR VERTEBRAL BONE DENSITY AND STRENGTH ASSESSED USING QCT-BASED FINITE ELEMENT ANALYSIS

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Conventional assessment of vertebral fracture risk has relied on areal bone mineral density of the lumbar vertebrae (L1-L4), even though vertebral fractures often occur in the thoracic region. Thus, we sought to investigate whether age-related declines in vertebral strength are similar in lumbar and thoracic vertebrae, and whether lumbar vertebral strength can be used to accurately predict thoracic vertebral strength. To do this, we used QCT-voxel based finite element analyses (FEA)(Keaveny et al., JBMR 2007) to assess vertebral strength in lumbar (L3) and thoracic (T10) vertebrae of 21 young (36 to 40 yrs) and 25 old (71 to 78 yrs) women from the Framingham Heart Study Offspring and Third Generation Multidetector CT Study. Mean compressive strengths of L3 and T10 vertebrae were both lower in old subjects than young subjects (4666±1274 vs. 10030±1654 N for L3; 4249±1360 vs. 7627±1702 N for T10), and were

associated with decreases in trabecular compressive strength of L3 and T10 of 65% and 54%, respectively (Table 1).

Table 1: QCT- and FEA-derived vertebral properties.	Average % Difference Young vs. Old	
	L3	**T10**
Compressive Strength	53.5*#	44.3*
Trabecular Comp. Strength	65.2*#	54.0*
Cortical Comp. Strength	36.9*	31.0*
Bending Stiffness	38.3*	25.7**
Axial Stiffness	46.4*	37.6*
Integral BMD	39.5*	34.1*
Trabecular BMD	44.2*	38.4*
Cortical BMD	29.4*	25.6*

*Young vs old: * $p < 0.0001$ ** $p < 0.005$
#Age by vertebral level interaction: $p < 0.01$

Interestingly, differences between old and young subjects were larger for L3 than T10 for all parameters measured, and the interaction between age and vertebral level was statistically significant for compressive strength and trabecular compressive strength ($p < 0.01$). The correlation between compressive strengths of L3 and T10 was moderately strong ($r2 = 0.54$ for both old and young women). Taken together, these data demonstrate a heterogeneity in vertebral strength across levels and over adulthood, due mostly to changes in the trabecular compartment. Accounting for this heterogeneity between vertebral levels may provide new insight into vertebral fracture etiology.

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BLUNTED SKELETAL RESPONSE TO PTH RETREATMENT IN MICE FOLLOWING AN INTERRUPTION IN DOSING

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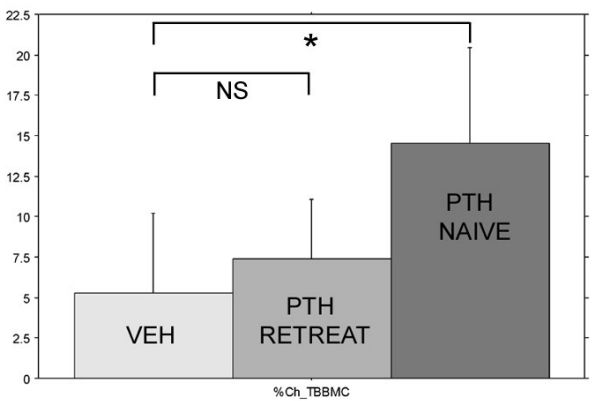
AIM: Teriparatide (hPTH (1-34)) is approved for the treatment of osteoporosis, but duration of therapy is limited to 2 years. Thus little is known about the skeletal response to interruptions in PTH treatment followed by retreatment. METHODS: We compared the skeletal response to PTH in retreated vs. treatment-naïve male C57Bl/6J mice. The retreatment group (RETREAT, N=4) received hPTH injections (40 ug/kg/day, sc, 5x/wk) from age 8-11 wks. Treatment was stopped from 11-17 wks of age and resumed from 17-20 wks of age. The treatment-naïve group (NAÏVE, N=4) received hPTH injections (40 ug/kg/day, sc, 5x/wk) from 17-20 wks of age only. Controls (VEH, N=8) received vehicle injections. Outcomes included bone densitometry (PIXImus) in vivo and on excised femurs.

RESULTS: We observed a significantly blunted anabolic response to retreatment with hPTH. From 17-20 wks, total body BMC increased significantly more than VEH (5.3 ± 5.0%) in the NAÏVE (14.5 ± 5.9%, $p = 0.02$), but not in the RETREAT (7.4 ± 3.7%, $p = 0.47$) group (see Figure). From 17-20 wks, total body BMC increased in PTH Naïve (+14.5 ± 5.9%, vs. VEH (+5.3 ± 5.0%) ($p=0.02$), but not in PTH Retreat (+7.4 ± 3.7%) vs. VEH (NS). All groups exhibited significant BMC increases vs. baseline ($p<0.05$), but PTH Naïve mice gained nearly twice as much total body BMC as PTH Retreat mice from 17-20 wks ($p = 0.087$). We then focused on the distal femur. Ex vivo, analysis of the distal 1/3 of the femur at 20 wks revealed 15-18% higher BMC in PTH Naïve vs. PTH Retreat ($p = 0.05$) or VEH ($p = 0.02$), but no difference between PTH Retreat and VEH.



CONCLUSION: These results suggest the skeletal response to PTH therapy is blunted after interruption in treatment, for reasons that remain to be elucidated. This phenomenon has major implications for osteoporosis therapy.

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PREDICTION OF FEMORAL STRENGTH IN A SIDEWAYS FALL CONFIGURATION USING QCT-BASED FINITE ELEMENT ANALYSIS

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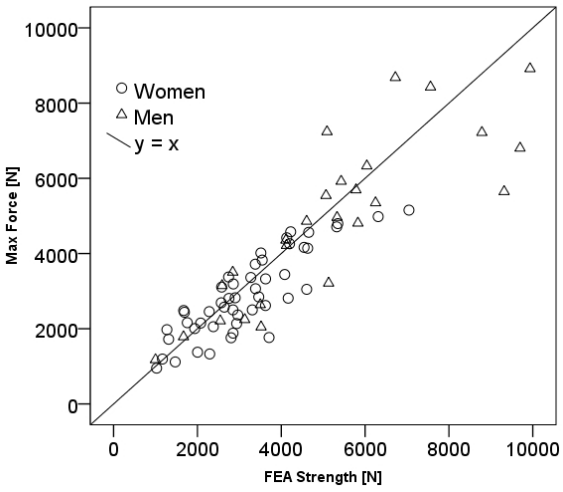
The gold standard for assessment of hip fracture risk is areal bone mineral density (aBMD, g/cm2) by DXA. However, recent studies have shown that up to half of those who fracture are not classified as osteoporotic by aBMD-based criteria. Thus, we sought to determine whether QCT-based Finite Element Analysis (FEA) is more strongly associated than aBMD with femoral strength. We obtained 76 human cadaveric femurs (50 female and 26 male, aged 74.2 ± 8.7 yr, range 55 to 98 yrs), measured femoral aBMD by DXA, acquired QCT images at 1 mm slice thickness, and used them to perform voxel-based FEA (ON Diagnostics, Berkeley, CA). Femora were then tested to failure at impact loading rate in a sideways fall configuration. Mean femoral failure load was 3573 ± 1821 N, and ranged from 950 to 8915 N. Both total hip aBMD and FEA were strongly correlated with failure load in the full set (r^2=0.72 and 0.78, respectively, Figure). To assess the ability of aBMD to *predict* femoral strength, the sample was divided into a "training" set (n=50) and "test" set (n=26). The regression between total hip aBMD and femoral strength developed in the training set was used to predict femoral strength in the test set. The mean error in predicting failure load was ±692 N (19.3% of the mean value). For FEA there was no training required, as the technique already provides a failure load prediction with no *a priori* knowledge. The mean error for FEA in the test set was ±702 N (19.6% of the mean value). In summary, these results show that with standardized imaging conditions and no soft tissue variation, femoral aBMD and QCT-based FEA are both strongly correlated with femoral strength in a sideways fall configuration. Further studies are needed to determine whether 3D-QCT based FEA will predict hip fracture risk better than DXA in clinical conditions where variability in subject positioning and body composition decreases the accuracy of aBMD measurements.



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MEASUREMENT OF CORTICAL THICKNESS IN ADOLESCENTS USING HR-PQCT

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We have experienced an evolution over the last decade in medical imaging tools that safely and precisely evaluate the growing skeleton and – most recently - characterize the hierarchical nature of growing bone. High-resolution peripheral quantitative computed tomography (HR-pQCT; XtremeCT, Scanco Medical™) permits *in vivo* (110 slices; 9.02mm) assessment of bone microstructure. It provides a direct assessment of cortical thickness (CTh, mm), an important component of bone strength. In the tibia, there is a rapid transition from highly trabecular bone and thin cortices at the metaphyses to wider cortices at the diaphyses. These characteristics are exaggerated in children where small trabeculae and thin cortices may present a measurement challenge. Therefore, our aims were, using HR-pQCT, to; 1) assess CTh in a defined region of interest (ROI) in the tibia and 2) use pQCT-based standards to determine whether the cortex could be assessed accurately across this ROI. We assessed CTh at the distal tibia (8% site) in 279 adolescents (133 girls and 146 boys; ages 16.7±1.7yrs old). Boys were 162.8±6.9cm tall, weighed 58.1±10.6kg and tibiae were 38.2±2.6cm long, on average. For girls, these values were 173.1±7.8cm, 66.2±14.5kg and 41.5±2.7cm. We used a customized method to assess a total of 11, 0.82mm sub-regions of interest (10 slices each; subROI). CTh increased 42% and 49%, on average from the most distal (1) to the most proximal (11) subROIs in girls and boys, respectively (Figure 1). We adopted the pQCT standard of ≥4 voxels per cortical ROI as accurate. Of the 279 participants, 97% had CTh that accommodated ≥4 voxels across the global ROI (110 slices) (Figure 2). Our results illustrate the substantial variation in CTh across a small ROI in the distal tibia. Although we were confident that we could assess CTh in this adolescent cohort, this may not be possible in more distal sites or in younger age groups.

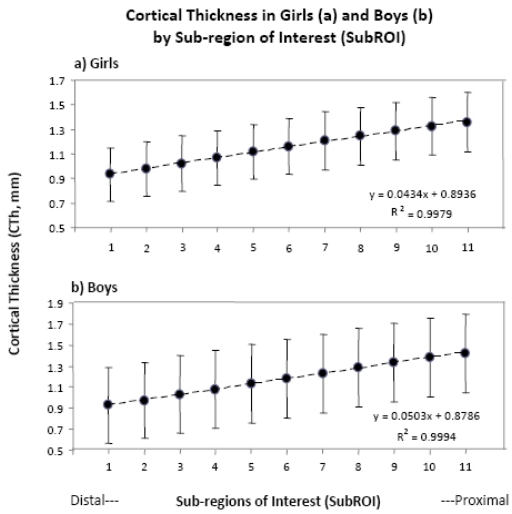

Figure 1: Cortical thickness (CTh, mm) by subROI in girls (a) and boys (b). Data are mean ± SD. Equations from linear regression are provided.

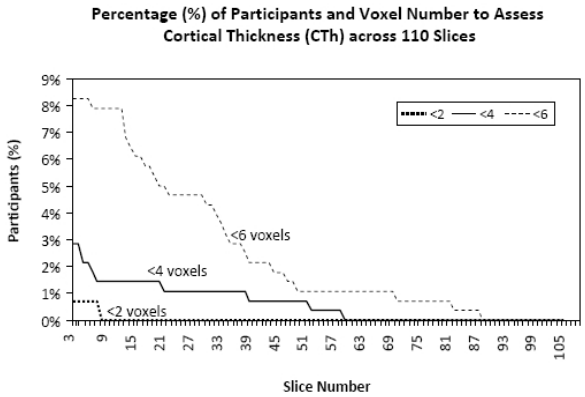

Figure 2: Illustrated are the percentage of participants whose cortices accommodated 2, 4, and 6 voxels or greater.

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THE MODE OF ACTION OF STRONTIUM RANELATE INVOLVES THE STIMULATION OF IGF-I PRODUCTION AND A DECREASE IN SIGNALS FOR OSTEOCLASTOGENESIS IN VIVO

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Strontium ranelate (SR) reduces vertebral and non-vertebral fracture risk in postmenopausal women. *In vitro* studies of primary human osteoblasts show that therapeutic doses of SR significantly increase OPG and reduce RANKL, thus reducing the signals for osteoclastogenesis. In addition, SR acts, at least in part, via the CaSR, and a recent study has shown that knocking out the CaSR interferes with local IGF-I and IGF-I receptor (IGF-IR) expression, and as such,

IGF-I signaling [1] . In male rats treated for 2 years with SR, serum IGF-I levels were significantly increased [2]. IGF-I is a known anabolic growth factor which enables skeletal development, growth and remodeling. To examine the early effect of SR on OPG, RANKL, IGF-I and IGF-IR *in vivo*, we treated 6 month-old mice with 1800mg/kg/day SR or vehicle for 2 and 4 weeks. SR led to 3.3+0.5 and 3.3+0.7-fold significant increases in local tibia mRNA levels of IGF-I at 2 and 4 weeks respectively compared to baseline control ($p<0.001$) and significant increases compared to their respective time-point, vehicle-treated controls ($p<0.01$ for both). The level of IGF-IR mRNA from the same tibiae was unchanged by SR. Circulating levels of IGF-I were significantly increased at 2 and 4 weeks compared to their respective time-point, vehicle-treated controls (both $p<0.05$). Tibia RANKL mRNA levels were significantly decreased by SR at 2 and 4 weeks, by 0.5+0.3 and 0.5+0.1–fold compared to baseline control ($p<0.05$ for both) and significantly decreased compared to their respective time-point, vehicle-treated controls ($p<0.05$ for both groups). OPG mRNA levels from tibia increased by 3.3+0.5 ($p<0.001$) and 3.9+0.6 ($p<0.001$) –fold at 2 and 4 weeks respectively compared to baseline control and significantly increased compared to their respective time-point, vehicle-treated controls ($p<0.001$ for both groups). SR also significantly increased BMD at 4 weeks compared to baseline ($p<0.05$), measured by m CT. These findings show that SR simultaneously increases circulating levels and expression of IGF-I in bone without affecting the receptor expression, thus increasing the anabolic IGF-I signal at the level of bone, as well as increasing tibia BMD after only a short, 4 week treatment period. In addition, SR reduces the signals for osteoclastogenesis *in vivo*, confirming previous *in vitro* findings and is in agreement with the dual mode of action of SR and the subsequent benefit on bone observed following SR treatment in post-menopausal osteoporotic women.

(1) Chang, W., et al., The Extracellular Calcium-Sensing Receptor (CaSR) Is a Critical Modulator of Skeletal Development. Sci. Signal., 2008. 1(35): p. ra1.

(2) Amman P., et al., Strontium ranelate improves bone resistance by increasing bone mass and improving architecture in intact female rats. JBMR, 2004 19(12):pp.2012-2020

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NEGLECT OF OCCULT VITAMIN D DEFICIENCY IN ACUTE HIP FRACTURE PATIENTS

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Background: Vitamin D deficiency is associated with increased risk of neck of femur fracture[1]. Low levels in Australian women is an independent predictor of falls, an important risk factor for fracture[2]. Overseas studies of acute hip fracture patients found a high prevalence of vitamin D deficiency with the majority of patients having levels below the desirable 75nmol/L. Australian data on this subject remains scarce.

Aims: To determine the prevalence of vitamin D deficiency and rates of vitamin D replacement in patients with acute hip fracture.

Methods: Two retrospective audits (2001/2002 and 2006/2008) were conducted at Austin Health as part of a quality improvement programme to improve osteoporosis management in patients with acute hip fracture. Data including calcium and vitamin D measurements and vitamin D supplementation were collected from the medical record, pathology and pharmacy databases. Intervention strategies to improve investigation and treatment of occult vitamin D deficiency from 2003 onwards included education of the Orthopaedic residents, liaison with the Orthopaedic and Rehabilitation Departments and implementation of a clinical pathway.

Results: Vitamin D deficiency ($\leq$ 50 nmol/L) was found in 92.8% of the pilot cohort and 66.5% of the follow-up cohort. Vitamin D requests increased by 2 fold in the follow-up audit (73.2% vs 33.3%, $p<0.0001$), and the rate of 25 (OH) vitamin D supplementation increased by 4 fold (43.7% vs 9.1%, $p<0.0001$). The mean dose of vitamin D supplementation did not differ according to vitamin D status ($p=0.066$), and the replacement dose was inadequate in 98.5% of patients. Only 1 out of the 467 patients in the follow-up cohort had hypercalcemia during admission.

Conclusions: Occult vitamin D deficiency is common amongst acute hip fracture patients in Melbourne. Vitamin D replacement was suboptimal and the replacement dose prescribed was mostly inadequate. Routine vitamin D replacement in acute hip fracture patient is safe, however a vitamin D level is still required to ensure adequate dosage.

(1) Weatherall, M. N Z Med J, 2000. 113(1108): p. 137-40.

(2) Flicker, L. J Am Geriatr Soc, 2003. 51(11): p. 1533-8.

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COMPARISON OF THE EFFECTS OF TERIPARATIDE AND CALCITONIN IN THE TREATMENT OF POSTMENOPAUSAL CHINESE WOMEN WITH OSTEOPOROSIS

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Aim: Vertebral fracture prevalence increases from 5% in Chinese postmenopausal women aged 50-59 years to 37% in those aged ≥80 years. We compared the effects of teriparatide (20 μg/day subcutaneously) or salmon calcitonin (200 IU/day intranasally) treatment on changes in surrogate markers of fracture risk (bone mineral density [BMD] and serum osteocalcin levels) to identify safe and efficacious osteoporosis treatments in this population.

Methods: This Phase 3 open-label, active comparator, randomized study in China enrolled 329 osteoporotic postmenopausal women (mean age: 68.7 years) with ≥1 previous fracture and a T-score of ≤-2.5 at the lumbar spine (LS) or total hip. Patients were randomized in a 2:1 ratio to teriparatide (n=220) or calcitonin (n=109) and treated for 6 months. Patients received supplements of calcium (≥500 mg/day) and vitamin D (200 or 400 IU/day).

Results: Baseline characteristics were generally well-balanced between treatment arms. The primary outcome was that teriparatide significantly increased LS BMD (adjusted mean difference [95% CI]) over calcitonin by 4.39% [2.89%, 5.89%; p<.0001]. The adjusted mean±SEM percent change from baseline to endpoint in LS BMD was 6.04%±0.48% with teriparatide (p<.0001) and 1.65%±0.65% with calcitonin (p=.0115). Changes from baseline and differences between treatments for total hip or trochanter BMD were not statistically significant. Median [IQ range] serum osteocalcin percent change from baseline to endpoint was increased significantly with teriparatide (126.87% [67.23%, 246.98%], p<.0001) and decreased with calcitonin (-12.34% [-25.70%, 7.33%], p=.0020); the between-treatment difference was statistically significant (p<.0001).

When compared with the calcitonin arm, more patients treated with teriparatide experienced adverse events and more events in the teriparatide arm led to discontinuation. The most commonly reported treatment-emergent adverse events were dizziness (7.4% teriparatide, 3.6% calcitonin) and muscle spasms (5.5% teriparatide, 3.6% calcitonin). Nine patients (8 teriparatide, 1 calcitonin) reported serious adverse events. One patient in the calcitonin arm experienced two new nonvertebral fractures; no new fractures were reported in the teriparatide arm. There were no clinically significant between-treatment differences observed in laboratory parameters.

Conclusions: Teriparatide resulted in significantly greater increases in lumbar spine BMD and serum osteocalcin compared with intranasal calcitonin in Chinese postmenopausal women. Both treatments were generally well tolerated.

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THE ROLE OF QUANTITATIVE ULTRASOUND OF THE CALCANEUS FOR DETECTING OSTEOPOROSIS IN PATIENTS WITH STRESS FRACTURES OF THE FEET

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Background: Stress fractures of the feet are a common occurrence. While there is a strong association between fragility fractures and low bone densitometry, the underlying pathogenesis of stress fractures of the feet may be more complex and often related to non-skeletal factors such as repetitive strain injury or bone fragility. Aim: To determine whether patients presenting with stress fractures of the feet have osteoporosis (localized or generalized). Methods: We measured lumbar spine (LS) and femoral neck (FN) BMD by dual energy X-ray absorptiometry (Lunar Prodigy) and

calcaneal stiffness (QUS) by quantitative ultrasound (Lunar Achilles) in 100 consecutive patients presenting with stress fractures of the feet (confirmed by either radionuclide bone scintigraphy or magnetic resonance imaging). T-scores were derived for each measurement from a normative database of normal healthy individuals. The precision for each of the measurements was 1.1%, 2.8% and 2.1% respectively. Data relating to risk factors for fractures were collected. Results are reported as mean + 1SEM and range. Comparisons were made using ANOVA). Logistic regression analysis was used to determine risk factors for fracture. Results: There were 86 women and 14 men with mean age 57.8 +1.4 years and weight 70.8+1.6 kg. Vitamin D deficiency occurred in 29%. Fractures were found in the bones of the left foot in 61% of cases. Osteoporosis (T-score < -2.5) was noted in 14% of patients using LS, 11% using FN and 42% using QUS ($P<0.001$ for comparison). Patients with talar dome and those with multiple fractures had the lowest QUS. The mean T-scores for LS=-0.7 (-4.2 to 0.2), FN=-1.1 (-3.3 to 2.0) and QUS=-2.2 (-4.8 to 0.8). The correlation between LS and QUS = 0.52 ($P<0.0001$) and between FN and QUS = 0.53 ($P<0.0001$). Age ($R=-0.48$; $P<0.0001$) and corticosteroid exposure ($R=-11.3$; $P<0.02$) were the major determinants of QUS. Conclusion: Feet fractures occur due to underlying localized osteoporosis, which can be detected more accurately using QUS.

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ALENDRONATE HAS AN ARRHYTHMOGENIC EFFECT ON CARDIOMYOCYTES *IN VITRO* BY AFFECTING CALCIUM DYNAMICS

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Therapy with bisphosphonates, including alendronate (ALN), is considered a safe and effective treatment for osteoporosis. However, recent studies have reported an unexpected increase in serious atrial fibrillation in patients treated with bisphosphonates (1). The mechanism that explains this side effect remains unknown. Since atrial fibrillation is associated with an altered sarcoendoplasmic reticulum calcium load, we studied how ALN affects cardiomyocyte calcium homeostasis and protein isoprenylation *in vitro*. Acute and long-term (48 h) treatment of atrial and ventricular cardiomyocytes with ALN (10-8-10-6 M) delayed and diminished subsequent calcium responses to caffeine. Only in atrial and not in ventricular cardiomyocytes, ALN induced transitory calcium oscillations acutely, and led to development of oscillatory component in calcium responses to caffeine following long-term ALN exposure. Long-term exposure of atrial cells to the lowest dose of ALN induced the most changes in calcium dynamics, including significantly higher frequency of calcium oscillations. Changes in calcium dynamics were accompanied by changes in expression of proteins controlling sarcoendoplasmic reticulum calcium. Sarcoendoplasmic reticulum ATPase 2 expression was increased in both atrial and ventricular cardiomyocytes. Calsequestrin expression was reduced in atrial cells but increased in ventricular cells. In contrast, ALN minimally affected protein isoprenylation in these cells. Our data indicate that treatment of atrial cardiomyocytes with ALN induced abnormalities in calcium dynamics and altered expression of calcium-handling proteins consistent with induction of a self-stimulatory, pacemaker-like behavior. These findings report a novel extracellular action of bisphosphonates, which may contribute to development of cardiac side effects associated with these drugs.

(1) Alendronate and atrial fibrillation. Cummings SR, Schwartz AV, Black DM. Engl J Med. 2007, 356:1895-6.

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BONE MINERAL DENSITY AT THE HIP IN NORWEGIAN WOMEN AND MEN. PREVALENCE OF OSTEOPOROSIS DEPENDS ON CHOSEN REFERENCES. THE TROMSØ STUDY

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Aim: This study describes bone mineral density (BMD) levels at the total hip and femoral neck in women and men between 30 – 89 years in an unselected population. The age specific osteoporosis prevalence was calculated based on the reference data provided by the Lunar Prodigy manufacturer and on the young adult mean in the study population.

Method: BMD was measured in g/cm² at total hip and femoral neck by dual-energy-X-ray absorptiometry (DXA), GE Lunar Prodigy, Lunar Corporation, Madison, WI, USA, in 3094 women and 2132 men in the 2001 Tromsø Study. Height and weight were measured in light clothing without shoes. The association between BMD and age was assessed by univariate analyses, and with multivariale analyses adjusting for covariates known to affect BMD levels. T-scores were calculated for each participant: measured BMD minus the young adult BMD divided by the standard deviation of the young adult BMD.

Results: BMD levels were significantly explained by age, and declined progressively in both genders from middle into old age, with the highest decline in women (Figure 1). In subjects above 70 years, predicted hip-fracture risk based on BMD differences was 2.7 times higher in women compared with men. With osteoporosis defined as a T-score of two and a half standard deviation below the young adult mean BMD, the prevalence at the total hip in subjects above 70 years was 6.9 and 15.3 percent in men and women, respectively, with T-score calculations based on the Lunar reference material. The prevalence increased significantly to 7.3 and 19.5 percent in men and women, respectively, with T-score calculations based on the young adult mean BMD (age group 30-39 years) in the study population. At the femoral neck, the corresponding prevalence increased from 13.5 to 18.5 percent in men, and from 20.4 to 35.2 percent in women (Figure 2).

Conclusion: Compared to the Lunar reference material, calculation of T-scores based on the population's young adult mean, resulted in higher osteoporosis prevalence which might reflect the higher fracture risk among Norwegians. Although BMD only partly explains fracture risk, future studies should evaluate which calculations give optimal fracture prediction.

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IS THERE HARD EVIDENCE FOR VERTEBROPLASTY / KYPHOPLASTY?

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The main goal of vertebroplasty and kyphoplasty is to relieve pain associated with vertebral compression fractures in a minimally invasive fashion. Up to date there has been no completed randomised controlled trials on this invasive procedure. The natural history of osteoporotic fractures has not yet been elucidated, thus the purported benefits of vertebral augmentation is still unknown.

Methods: This is a systematic review of all the available data presented in peer-reviewed published clinical trials on Medline. The methodological quality of included studies was evaluated and data were collected targeting specific measurements.

Results: The majority of studies in the literature presented retrospective findings. The success rate in pain relief was 70–90%. Correction with kyphoplasty was often limited (less than 15°) and has not been shown to increase pain relief or quality-of-life compared to vertebroplasty. The rate of radiculopathy was 4% and the rate of cord compression was less than 0.5%. Cement leaks occurred in 41% and 9% of treated vertebrae for vertebroplasty and kyphoplasty respectively. The odds ratio of a vertebral fracture in the vicinity of a cemented vertebra was 2.27 (95% confidence interval 1.1–4.56), compared with 1.44 (95% confidence interval 0.82–2.55) for a vertebral fracture in the vicinity of an uncemented fractured vertebra.

Conclusions: There were difficulties in comparing the different studies and evaluating the endpoints as the setup and documentation of the studies differed from each other. The procedure has a low rate of clinical complications, but the potential complications can be devastating. To evaluate the efficacy of percutaneous vertebroplasty requires prospective controlled trials with long-term follow-up.

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MISSED TREATMENT OPPORTUNITIES: A PROSPECTIVE OBSERVATIONAL COHORT STUDY OF PATIENTS ADMITTED WITH HIP FRACTURES.

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Background: Mortality associated with hip fractures is 33%, of which 95% are attributable to OP. Despite the data about prevention, the incidence of preventable hip fractures is on the rise with an increase of 106% by 2021. [i]

Objectives: To demonstrate the management of patients with previous fractures and falls, who now present with a hip fracture.

Design: A prospective observational cohort study over 8 consecutive months, undertaken in a metropolitan tertiary hospital with two orthopaedic units and emergency department.

Cases: All patients admitted with hip fractures due to minimal trauma over a period of 8/12 (n = 106).

Method: Patients were seen on admission and assessed prospectively. Data collected consisted of a detailed past history, including previous falls and fractures prior to this presentation, and the management of osteoporosis and falls prior to admission..

Results: There were a total of 106 patients identified who were eligible for inclusion in this study. Of those patients, 34%(n=36) had a history confirming a diagnosis of OP; that is, 24.5%(n=26) had sustained a previous fragility fracture, and 9.5%(n=10) had been diagnosed with OP via DEXA scan. Of the patients with OP, 59% were not on an anti-resorptive agent and had no falls prevention measures instituted. Only 14%(n=5) were on the recommended and indeed ‘optimal combination therapy' of anti-resorptive agent plus Calcium and Vitamin D supplementation.

This lack of treatment was even more pronounced in those with previous fractures of which only 8% were on anti-resorptives and 27% were treated with Vitamin D and Calcium supplementation.

Conclusion: Despite patients presenting in the community with falls, fractures and a diagnosis of osteoporosis , management has been suboptimal. Access to health services for prevention measures and compliance issues require further review.

(1) Pockock, N. et al. 'The potential effect on hip fracture incidence of mass screening for osteoporosis '. MJA 1999; 170: 486-488

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DISABILITY IN PATIENTS WITH OSTEOPOROTIC PERTROCANTHERIC HIP FRACTURE: A PROSPECTIVE STUDY

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Purpose: To study the short-medium term (6 months) disability associated to the stable osteoporotic pertrochanteric hip fracture.

Methods: Prospective study, with inclusion, during a two-year period (March 2002 - December 2003), of all patients older than 65 years with stable osteoporotic pertrocantheric hip fracture (Kyle I-III) who were attended in a 400-bed hospital in Spain. Patients with a moderate-severe disability (impossible to walk by themselves or Barthel <60) prior to hip fracture were excluded. Functional status was evaluated at inclusion (retrospective measurement of disability prior to fracture), and during follow-up (1,3 and 6 months) using: Barthel Index (0-100), the Activities of Daily Living index (ADL, 0-6), and the evaluation of the Walking Ability (WA) by a validated method that gather the need for walking aids.

Results: 82 patients (90% women) with a mean age of 83±7 years were included. The functional status at inclusion was: Barthel index 61±22,6; and 44% and 68% of the patients were independent according to ADL and WA scales respectively (table 1). Disability clearly improved during the follow up, but still remained very important after 6 months (table 1). At six months 37 patients (45%) were lost.

Conclusions:

Hip fracture, even the most stable type, produces an important disability at medium term that is not always taken into account in these patients.

Baseline	1 month	3 months	6 months	
Barthel Index	88,3±10,8	61±22,6 75	75,9±22,4	85,4±15,7
ADL scale*	44%	3,5%	18%	26,5%
WA scale**	68%	0%	9%	21%
*Independent patients for the 6 basic ADL. **Independent patients for walk				

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SEXUAL DIMORPHISM IN BONE FRAGILITY IS PARTLY DUE TO THE DIFFERING INTRACORTICAL REMODELLING AND POROSITY

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The morphological basis of sex differences in bone fragility is uncertain. Larger bone size and better maintenance of trabecular connectivity may be partly responsible. However, little attention has been given to cortical bone even though 80% of bone is cortical. Age-related increase in intracortical porosity erodes the cortex adjacent to the marrow producing a large proportion of the bone loss, cortical remnants having a trabecular appearance ("trabecularization") and cortical thinning from 'within'. As remodelling is slower in men than women, at least until late in life, we speculated that the age related increase in cortical porosity and thinning occur later in men and the spurious constancy in trabecular density (BV/TV) observed using QCT produced by cortical remnants will be seen later in men. We studied 54 men (aged 52-98 yr) and 211 women (aged 50-99 yr) using HR-pQCT at the distal radius. In both sexes age-related diminution in cortical thickness, cortical density and BV/TV was detectable after 50 yrs. In men, correlations between cortical thickness, cortical density and BV/TV with age were respectively -0.59 ($p < 0.01$), -0.63 ($p < 0.01$) and -0.27 ($p = 0.04$). The corresponding figures in females were -0.49 ($p < 0.01$), -0.57($p < 0.01$) and -0.15 ($p = 0.03$). The age-related diminutions in cortical thickness and density remained in both sexes aged 50+, 60+, and 70+. The correlation between BV/TV and age were no longer significant in 131 women 60 years and older ($r = - 0.12$; $p = 0.2$) but remained significant in the 19 men (despite the small number of men) ($r = - 0.32$; $p = 0.05$). After the age of 70, BV/TV did not diminish with age in either sex. These observations are consistent with the hypothesis that the intracortical porosity producing cortical remnants spuriously maintains BV/TV in the 7^{th} decade in men and 6^{th} decade in women. We infer that sexual dimorphism in fragility is partly the result of differing intracortical remodelling and porosity.

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NON-VERTEBRAL FRACTURE RISK OF RISEDRONATE TREATED PATIENTS IS SIMILAR TO THAT OF UNTREATED PATIENTS 10 YEARS YOUNGER

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Non-vertebral fractures represent 73% of all osteoporotic fractures, and greater than 90% of all osteoporosis-related fracture costs1. Age is a well known independent risk factor for fracture. Older patients with lower BMD T-scores are at increased risk for fracture. In this research, we examined the effect of risedronate treatment on non-vertebral fracture risk as a function of patients' age.

Postmenopausal osteoporotic women (n=3229) who were enrolled in risedronate clinical trials (VERT, RON & ROE) who took at least one dose of placebo or risedronate 5mg pill were included in the analysis. Patients (age:38-85 years) had a mean FN T-score of -2.2.

To assess the impact of age on fracture risk, Cox regression model with age and treatment as explanatory variables was used. Both treatment and age had a statistically significant impact on fracture risk. The interaction between treatment

and age was included in the initial model but removed from the final model as it was not statistically significant (p>0.05).

The association of the increased risk for fracture and age did not differ significantly by treatment groups (p>0.05 for the interaction term). Regardless of treatment group, for every 1 decade increase in age, patient risk for any osteoporotic non-vertebral fracture increased approximately 68% on average. Risedronate reduced the fracture risk by 41%, p<0.001 after adjusting for age, suggesting that the magnitude of the treatment benefit was consistent regardless of age.

When comparing 3-year fracture risk between risedronate and placebo patients, we found that fracture risk for patients who had been treated with risedronate 5 mg was estimated to be similar to the placebo patients who were 10.3 years (95% CI: 4.4, 20.2) younger in age. Seventy year-old patients in the risedronate 5 mg group had a risk of fracture similar to sixty year-old patients in the placebo group.

In conclusion, every decade increase in age is associated with an increase in nonvertebral fracture risk by about 50%. In patients treated with risedronate, nonvertebral fracture risk was reduced to the risk seen in untreated patients who were 10 years younger in age.

(1) Burge R, et al. J Bone Miner Res. 2007;22:465-475.

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USE OF ZOLEDRONIC ACID IN REAL WORLD. FIVE YEARS EXPERIENCE

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Zoledronic acid has recently been authorised for the treatment of postmenopausal osteoporosis. Strong evidence supports its antifracture efficacy but several concerns with regard to safety aspects had been aroused.

In our experience, IV Zoledronic acid has demonstrated to be an effective antiosteoporotic treatment reducing bone biochemical metabolic markers and increasing BMD in patients suffering from osteoporosis that had previously been treated with other antiosteoporotic treatments. Zoledronic acid has shown a good safety profile: 306 patients were included from years 2003 to 2008. Seventy-five percent of them were women. Seventy percent of them suffered from secondary osteoporosis. Mean age was 69+/- 11 years. All of them were previously treated with at least, two antiosteoporotic treatments. The total number of Zoledronic acid infusions was 612. Twenty-six patients received 4 infusions. The basal values of BAP were 14,2+/-8 and 50+/- 28 of NTX. Lumbar spine BMD was 0,757+/-0,134 and femoral neck BMD was 0,679+/-0,129. BAP and NTX values significatively decreased after the first infusion (37% and 47.5% respectively). Lumbar spine BMD values significatively increased after the first year, 3,6% (n=60, p<0.001). No statistically significative increase was found in the following years. Femoral neck BMD increase at two years was 3,4% (n=30, p<0.001). Recorded adverse events after 612 infusions were arthromyalgias (4,11%); arterial hypertension during infusion (0,5%); uveitis (0,33). No cardiac arrythmias or bone necrosis were found. Patients that had been treated with at least one IV infusion of Zoledronic acid, were included. All of them received it for the treatment of osteoporosis. Zoledronic acid was used after the failure or intolerance to other antiosteoporotic treatments such as; Bisphosphonates, Raloxifene and others. Data on efficacy were recorded: Changes in serum levels of bone biochemical markers (Bone specific alkaline phosphatases (BAP) and N-telopeptides in urine (NTX); Changes in BMD (Lumbar spine and Femoral neck). Adverse events were also recorded..: To present our experience in the use of IV Zoledronic acid in osteoporotic patients.

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SUBTROCHANTERIC STRESS FRACTURES IN SIX PATIENTS ON LONG TERM BISPHOSPHONATE THERAPY : A CASE SERIES.

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There is increasing concern that long term bisphosphonate therapy may over suppress bone turnover thus limiting microdamage repair and predisposing the patient to stress fractures. Growing numbers of reports are emerging in the literature of patients on long term bisphosphonate therapy experiencing subtrochanteric stress fractures with characteristic radiological and clinical features.

We describe our experience in an Orthogeriatric Unit at a tertiary hospital in Western Australia where we identified and reviewed six such cases over a 12 month period. All were female with their ages ranging from 60 to 87years. To our knowledge, all reports to date have been associated with Alendronate therapy. We describe the first case in the literature to be potentially associated with Risedronate therapy. Duration of therapy ranged from 18 months to 16 years. Four had prodromal pain ranging from 1 week to 6 months suggestive of a stress fracture and two presented with spontaneous fracture with no antecedent fall. One case had bilateral fractures. (See Table 1.)

	AGE	BISPHOSPHONATE	DURATION	FRACTURE CIRCUMSTANCES	PROPROMAL PAIN
CASE 1	85	Risedronate	At least 3 years	Spontaneous fracture	Unable to determine
CASE 2	76	Alendronate	18 months	Fall	6 months
CASE 3	80	Alendronate	8 years	Fall	1 week
CASE 4	87	Alendronate	8 years	Fall	5 months
CASE 5	60	Alendronate	8 years	Fall	1 month
CASE 6	74	Alendronate	16 years	Spontaneous fracture	6 months (bilateral)

Table 1: Clinical Features

Their biochemistry including serum calcium, Vitamin D and bone turnover markers are described.

Radiology in these patients was similar to that reported in the literature. The typical transverse fracture with unicortical break and cortical hypertrophy is seen in all with one case showing a stress fracture in the contralateral limb as well.

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RECRUITMENT STRATEGIES FOR AN OSTEOPOROSIS CLINICAL TRIAL

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Competitive recruitment for clinical research studies has become the norm. Short recruitment windows are also expected in most research studies. We organised our recruitment strategies for an osteoporosis clinical trial over an 8 week period. The following recruitment methods were used; radio and newspaper advertising, database searches of bone density and xray results, specialist and primary practice databases. Study specific posters were placed in convenient venues along with community initiatives, displays and community meetings. A site specific pamphlet was also generated. This resulted in a total of 246 subjects being screened, 41 eventually consenting to the study and 14 going on to be randomised. A total of 191 were screen failures, ineligible or declined to participate.

In summary the most effective rapid recruitment methods appeared to be in-house database searches, newspaper and radio advertisements. The least effective were poster displays, doctor contacts, talks and community initiatives.

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EFFECT OF VEGETARIAN DIET ON BONE MINERAL DENSITY: A BAYESIAN META-ANALYSIS

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The association between daily diet and bone mineral density (BMD) is controversial, due to conflicting findings from previous studies. This study sought to estimate the magnitude of effect of vegetarian diets on BMD by using a meta-analytic approach.

A systematic electronic search of literature was conducted to identify all published studies in English on the association between diets (vegetarian and non-vegetarian) and BMD. In total, 9 studies were included in the analysis, involving 2749 individuals (1880 women and 869 men). Traditional and Bayesian methods of meta-analysis were applied to synthesize the data.

Overall, compared to omnivores, vegetarians had lower BMD by about 4% (95% CI: 2% to 7%) at the femoral neck and lumbar spine. The effect was more pronounced in Caucasians (among whom BMD in vegetarians was lower than omnivores by between 8% and 10%) than in vegetarian Asians among whom the difference was between 2% and 3%.

The probability that BMD of vegetarians is higher than omnivores by at least 5% (or ~0.3 SD) was 0.42 for the femoral neck and 0.32 for the lumbar spine. The probability that BMD of Caucasian with vegetarian diet is higher than their omnivorous counterparts by at least 5% was 0.56 for the femoral neck and 0.75 for the lumbar spine.

These results suggest that vegetarianism is associated with lower bone mineral density, but the effect is unlikely to be of clinical significance.

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VERTEBRAL FRACTURES IN ADULTS WITH AN INTELLECTUAL DISABILITY.

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Adults with an intellectual disability (ID) have increased prevalence of osteoporosis,[1] but also difficulty in accessing bone densitometry. Identification of vertebral fractures on X-ray assists in identifying reduced bone integrity.[2] However, the diagnosis can commonly be missed because only one-third of vertebral fractures are symptomatic. Additionally, adults with an ID have difficulty describing their pain.[3]

The prevalence of vertebral fractures in adults with an ID is relatively unknown. This study asked whether the prevalence of vertebral fractures in adults with an ID is greater than the general population; and if undiagnosed vertebral fractures are more common in study participants with communication difficulties. Participants were aged 18 or over and had an ID. Modified quantitative analysis (diagnostic threshold: ≥20% vertebral height loss) of existing lateral spine or chest radiographs was performed.

Of 65 participants (37 men, 28 women) (mean age 51 years), 30 (46%) had one or more vertebral fractures. Both this increased prevalence, as well as the mean number of vertebral fractures per participant (0.8), were higher ($p<0.0005$) than the general population.[4] A multiple regression model was identified which comprised: use of anti-epileptic medicaiton; having had any type of prior fracture; and being able to verbally localize pain symptoms. Age was not associated with increased numbers of vertebral fractures ($p=0.33$). Previously undiagnosed vertebral fractures were more common in participants (n=20) who were unable to verbally localise their pain ($p=0.056$).

Further research on reasons for, and prevention of, vertebral fractures and reduced skeletal integrity in adults with an ID is indicated. Clinical emphases given to reporting fortuitous radiographic findings of vertebral fractures and of noting non-verbal indications of pain could reduce the risk of non-diagnosis.

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Z-SCORE COMPARISON OF BONE DENSITY REFERENCE DATABASES IN CHILDREN

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Several reference databases for different dual-energy x-ray absorptiometry (DXA) machines (brand, model, software) serve interpretation of bone density (BMD) results in children. The diversity of DXA-scanners and reference databases questions if the choice of the reference database generates greater differences in the interpretation of the measured

bone density results than expected. This study aimed to compare all currently available paediatric DXA reference databases on Hologic®-scanners, applied on BMD-results of a large series of unselected patients.

2027 DXA-scans of paediatric patients were extracted from Hologic®-QDR-4500A machines at our institutions. Age- and sex-specific BMD-z-scores, calculated according to sex-specific equations from six published international databases [1-6] , were compared for the lumbar spine (LS) femoral neck (FN), total hip (TH), and total body (TB). Analysis was restricted to the age-range between 8-17 years, which was covered by most databases.

The final dataset included 1313 scans (772 of girls). Average z-scores differed significantly ($p<0.001$) in each scan region (except for FN male), in particular the TB (see table).

	Sex (n)	Kalkwarf (1)	Ward (2)	Kelly (3)	Ellis (4)	Bachrach (5)	Faulkner (6)
Hologic-Model		QDR-4500A & W, Delphi-A	QDR-4500A	QDR-4500A, Delphi-A	QDR-2000W	QDR-1000W	QDR-2000
		Median (IQR 25th to 75th)					
LS	**F** (201)	-0.94 (-1.92 to -0.21)	-0.93 (-1.78 to -0.08)	-1.09 (-2.08 to -0.40)	-0.95 (-1.71 to -0.27)	-1.35 (-2.12 to -0.74)	-0.95 (-1.71 to -0.27)
	M (106)	-0.73 (-1.60 to -0.13)	-1.06 (-1.96 to -0.26)	-0.81 (-1.46 to -0.17)	-0.80 (-1.43 to -0.05)	-1.22 (-1.90 to -0.50)	-0.80 (-1.43 to -0.05)
FN	**F** (202)	-1.10 (-1.79 to -0.21)	-0.68 (-1.64 to 0.14)	-	-1.01 (-1.69 to -0.23)	-1.02 (-1.60 to -0.37)	-1.01 (-1.69 to -0.23)
	M (101)	-0.89 (-1.75 to 0.08)	-1.19 (-1.96 to -0.34)	-	-1.04 (-1.79 to -0.18)	-1.13 (-1.96 to -0.11)	-1.04 (-1.78 to -0.18)
TH	**F** (201)	-1.23 (-1.98 to -0.24)	-1.16 (-2.08 to -0.01)	-1.16 (-1.84 to -0.26)	-	-1.08 (-1.69 to -0.19)	-0.98 (-1.76 to -0.12)
	M (101)	-1.07 (-2.05 to -0.13)	-1.30 (-2.16 to -0.61)	-1.02 (-1.76 to -0.26)	-	-1.26 (-2.10 to -0.56)	-0.76 (-1.64 to 0.12)
TB	**F** (168)	0.39 (-0.40 to 1.17)	-0.05 (-0.67 to 0.71)	0.29 (-0.42 to 1.11)	-0.15 (-0.66 to 0.45)	0.16 (-0.34 to 0.70)	0.64 (0.03 to 1.45)
	M (233)	0.45 (-0.30 to 1.20)	-0.19 (-0.81 to 0.52)	0.30 (-0.37 to 0.94)	-0.38 (-0.94 to 0.32)	0.58 (-0.18 to 1.25)	0.57 (-0.11 to 1.45)

Although z-scores calculated from different databases were highly correlated, there were significant differences between z-scores. Our results indicate that interpretation of DXA-results is only as good as the reference database used, including the possibility of false diagnosis and unnecessary therapies. This study was not designed to determine the optimal reference database and there are other differences in children's bone mass, shape, strength and body size that are undetectable by DXA. Ideally, z-scores should be calculated using model-, brand-, and software-specific reference curves for age, sex and ethnic group.

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EFFECTS OF *PIPER SARMENTOSUM* ON BONE RESORPTION AND ITS RELATIONSHIP TO PLASMA CORTISOL IN RATS.

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Osteoporosis is a proven complication of long-term glucocorticoid therapy. High levels of serum glucocorticoids inhibit bone formation and increase bone resorption activity. Glucocorticoids also inhibit calcium absorption from the intestines and increase calcium loss via the kidneys.

Piper sarmentosum is an erect herb with long creeping stems. It has long been used by villagers as a traditional remedy for cough, fever, toothache and fungal skin infection. The methanolic extract of *Piper sarmentosum* possessed a natural antioxidant, naringenin, belonging to the superoxide group. Our own previous studies have shown that Piper sarmentosum has the ability to inhibit the 11- β -hyroxysteroid dehydrogenase (11- βHSD) enzyme, an enzyme important in the synthesis of cortisol. Based on this and also its antioxidant property, we hypothesize that *Piper sarmentosum* will be able to prevent osteoporosis in a rat model of excess glucocorticoids.

Forty male Sprague-Dawley rats, aged 3 months and weighing between 200-250 g were used. Twenty-four animals were adrenalectomized and replaced with intramuscular dexamethasone 120 μg/kg/day. They were simultaneously

given either *Piper sarmentosum* (*Piper s.*) 0.125g/kg/day, glycyrrhizic acid (GCA) 240 μg/kg/day or vehicle distilled water (adrx-control group) daily by oral gavage. A group of eight animals were sham-operated and given vehicle daily, i.e. intramuscular olive and oral distilled water. The treatment was given for 8 weeks. The group given GCA was used as a comparison since GCA had been proven to inhibit 11- βHSD enzyme, therefore reducing serum cortisol levels. A baseline control group was used to determine whether any significant stress was induced in the sham-control group.

The results after eight weeks showed increased serum cortisol levels in the adrx-control group compared to the sham-control group. However, in the groups treated with *Piper s.* and GCA, the serum cortisol levels were maintained at sham-control levels. Plasma pyridinoline (bone resorption marker) levels were lower in the *Piper s.* and GCA groups compared to the adrx-control group. No difference in serum osteocalcin levels were detected between all the groups.

In conclusion, the results showed that *Piper s*. was as effective as GCA in preventing the increased cortisol levels seen in adrenalectomised rats treated with dexamethasone. This was associated with reduced bone resorption activity. Therefore, this suggests that *Piper s*. is potentially useful in prevention of osteoporosis induced by long-term glucocorticoid therapy.

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THE IMPACT OF AN OSTEOPOROSIS CLINICAL GUIDELINE ON RATES OF REFERRAL TO AN OSTEOPOROSIS CLINIC

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Purpose: Osteoporosis is under recognised and under treated in the tertiary setting. In response, Area Rehabilitation and Aged Care commenced a service to provide investigation and management services to post-minimal trauma fracture (MTF) patients. The clinic, named the Fragile Bone Clinic, was designed to review patients aged 65 and over who were discharged directly from the Emergency Department (ED). Despite establishment of the Fragile Bone Clinic, referral rates remained low, with 20 referrals received during 2006 and 2007 combined.

Methods: A clinical guideline on investigation and management of patients post- MTF was developed by Area Rehabilitation and Aged Care staff, in consultation with clinicians interested in osteoporosis, and widely disseminated throughout the hospital in late 2007.

Results: Between 1st January 2008 and 30th September 2008, 37,854 patients presented to the tertiary ED. Of these patients, 30% were aged 65 and over. In this age group 5% of patients were considered to have MTF.

Of the 569 MTF patients aged 65 and over, 194 (34%) were eligible for referral to the Fragile Bone Clinic. Of the patients eligible for referral to the clinic, 26% (51) were referred. When patients were offered review at the clinic 84% accepted an appointment.

During the period 01/01/2008- 30/09/2008:	Number	Percent
Patients reviewed in the Emergency Department	37,854	
Patients aged 65 years and over reviewed in the Emergency Department	11,436	30
Patients aged 65 years and over with a MTF	569	5
Patients eligible for referral to the Fragile Bone Clinic	194	34
Patients referred to the Fragile Bone Clinic	51	26
Patients reviewed in the Fragile Bone Clinic	43	84

Conclusion: Implementation of a clinical guideline for investigation and management of MTF patients resulted in an increased rate of referral to an Osteoporosis Clinic. This suggests that, among other strategies, the presence of a clinical guideline may increase rates of osteoporosis awareness and referral by clinicians.

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OSTEOPOROSIS SURVEY: GP MANAGEMENT POST FRAGILITY FRACTURE

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Purpose: Patients with fragility fracture secondary to osteoporosis are at risk of recurrent fracture. Osteoporosis (OP) is often under recognised and under treated. A previous study (Inderjeeth et al, IMJ 2006), confirmed low rates of awareness, investigation and treatment following fragility fracture. We surveyed General Practitioners (GPs) to assess their attitude to osteoporosis investigation and treatment following a fracture.

Methods: GPs were invited to complete a web based survey (Survey Monkey). The survey included questions on GPs attitude to review, informing, investigating, treatment, follow up, referral and responsibility for care of patients following a fragility fracture.

Results: A total of 306 GPs have responded so far. Their approach to managing their patients following a fracture are summarised in the table below. There were concerns about short and long term side effects of bisphosphonates (22% and 32%), Strontium Ranelate (27% and 31%) and Raloxifene (20% and 29%). There was a significant association between GP review on discharge and Investigation and initiation of OP treatment ($p<0.05$). There was a significant association between the initiation of specific OP treatment and concern re short term side effects of bisphosphonates and Strontium Ranelate ($p<0.05$); but not the long term concerns.

GPs who Always or Mostly:	Percent
Review patients on discharge from hospital	78
Inform patients they have OP	84
Request a BMD to assess patients regularly	42
Their responsibility to investigate patients for OP	85
Commence Calcium supplements	83
Commence Vitamin D supplements	67
Commence Specific OP treatment	73
Consider “other doctor” responsible for OP	22
Refer patient to “other doctor or clinic”	7

Conclusion: This survey suggests higher rates of review, investigation and treatment of patients by GPs following fragility fracture. This is at odds with published data on rates of patient awareness, investigation and treatment. The reason for this discrepancy may be important in understanding and improving OP treatment and fracture prevention. It is possible that the respondents in this survey may have responded due to a bias towards an interest in OP management.

(1) Inderjeeth CA et al, Intern Med J. 2006;36:547-551

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AN AUDIT OF USE OF ZOLEDRONIC ACID IN PATIENTS WITH OSTEOPOROSIS IN A TERTIARY INSTITUTION

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Purpose: Zoledronic acid has been demonstrated to reduce bone turnover, improve bone mineral density and reduce fracture rates. Side effects are reported to occur albeit infrequently. We report our data on 131 mainly older patients given a Zoledronic acid infusion to manage their osteoporosis. Most had been changed over from Pamidronate or other anti-resorptive treatments.

Method: We prospectively collected demographic, clinical and result of investigations and information on side effects reported following the infusion. Patients were phoned by a nurse 1 week post-infusion and a proforma completed on side effects reported. Data collected included baseline fractures, new clinical fractures reported between infusions, biochemical markers pre- and post-infusion, and BMD results where available.

Results: The mean age of the study population was 79.5 years (SD 9.37). A total of 131 patients had between 1 and 3 infusions. At 12 months, 15.3% reported a new clinical fracture, and at 24 months, 10% reported a new clinical fracture. The most common site of fracture was the vertebrae. The mean N-telopeptide/creatinine ratio (NTx/Cr) pre-

baseline infusion was 35.78 ±16.13nmol/mmol, pre-12-month infusion was 28.67 ±14.74nmol/mmol and pre-24-month infusion was 25.20 ±6.98nmol/mmol. The interval reduction was not significant, with a p-value of 0.113 and 0.325 respectively. However at baseline, 40% had NTx/Cr <30nmol/mmol and 20% <20nmol/mmol compared with 77% and 59% respectively at 12 months. The mean Alkaline phosphatase (ALP) reduced from 95.94 ±46.82U/L to 75.94 ±42.17U/L at 12 months (p-value 0.171). Following the baseline infusion, the mean total calcium decreased from 2.38 ±0.12 mmol/L to 2.32 ± 0.11 (p-value <0.001) 10-14 days post-infusion. Ionised calcium, magnesium, phosphate, creatinine and potassium did not change significantly. Mean vitamin D pre-infusion was 67.19 ±22.52nmol/L. Limited BMD data showed variable changes at the spine and hip. Side effects reported were similar to published data except for fever (24%) and arthralgia (16%) at baseline, with significant reduction (50%) following subsequent infusions.

Conclusion: Zoledronic acid appears to be reasonably well-tolerated in most older patients. Apart from a reduction in serum total calcium, other electrolytes were not affected. A high proportion of patients had persistently suppressed bone-turnover markers at 12 months.

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COMPARISON BETWEEN TEN-YEAR FRACTURE RISK ASSESSMENT BY THE NORLAND ILLUMINATUS SYSTEM AND WHO BASED TEN-YEAR FRACTURE RISK ASSESSMENTS

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Ten-year fracture risk assessment involves a review of clinical risk factors with age and bone density and is showing increased clinical interest. Several processes of making the assessment are, however, available and it is of interest to see how these different approaches impact the assessment.

A population of 75 women between 35 and 85 years of age and a Femur Neck T-score between -3.90 and +0.38 underwent a clinical history and DXA scan with a Norland XR-46 to prepare a Ten-year Fracture Risk Assessment. Ten-year Fracture Risk Assessments were prepared using: 1) an Illuminatus based program, 2) a Paper Chart FRAX™ WHO Fracture Risk Assessment Tool system (PCS), 3) a FRAX™ Patch T-score and the Paper Chart System based assessment (PPCS), 4) a FRAX™ Patch T-score with a Body Mass Index of 24.0 using the FRAX® WHO Fracture Risk Assessment Tool (24FRAX) and 5) an assessment with the FRAX™ Patch T-score and the patient's Body Mass Index using the FRAX® WHO Fracture Risk Assessment Tool (PFRAX). Assessments were compared to determine if conclusions were similar.

Significant positive correlations with differences in slope were seen between Ten-year Fracture Risk assessed by the Illuminatus based assessment and the other assessments. Applying criteria for treatment as an Osteoporotic T-score or a Ten-year Fracture Risk higher than 3.0% to the population, concurrence of treatment between the Illuminatus based assessment and PCS was 98.7%, PPCS was 88.0%, 24FRAX was 81.3% and PFRAX was 86.7%.

In conclusion, Ten-year Fracture Risk Assessment can generate consistent results. A number of analysis routines, however, are available in the market and the clinician should be aware that some differences will exist between assessments. Use of Ten-year Fracture Risk Assessment should take into account how the Ten-year Fracture Risk Assessment was performed.

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SHARED DECISION MAKING IN OSTEOPOROSIS : IMPROVEMENT THROUGH EDUCATION

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Osteoporosis is a chronic disease, and shared decision making (SDM) is essential to ensure long term adherence to therapy. This work assesses the effect of a brief educational intervention in changing attitudes of patients towards SDM. A questionnaire was administered to 120 subjects with osteoporosis. 50 subjects were then referred to a trained dietician for counseling in diet, activity, architectural remodeling, FRAX tool, and need for therapy, over 4 weekly sessions of 30 minutes each, followed by readministration of the questionnaire. Of the 120 subjects, 104 were female,

(average age 62.5 years). 85.8% realized osteoporosis could cause fracture; 21.7% knew it could cause hospitalization,4.2% felt it could permanently disable, and 3.3 % thought it could kill. All subjects said they would like to be consulted/informed if an antiosteoporotic medication was added to their prescription. 100.0% were willing to take calcium , 65.5% felt they could increase physical activity, and 25.0% thought domestic architectural remodeling was possible.100.0% agreed upon the need to take oral medication while 33.3 % were open to of injectable therapy 76.7 % said they would accept 1-2 tablets/day for osteoporosis, while 23.3% were comfortable with 3 tablets.¬¬ Only 28.3% were ready to take a daily injection, while 33.3% were happy to accept an injection once in three months, and 15.0% were comfortable with a nasal spray. In the intervention group, after 1 month, the proportion of subjects willing to accept physical activity / physiotherapy rose to 92.0%, architectural remodeling increased to 34.0% and 42.0% felt it was worth taking daily injectable therapy. 100% were comfortable with a prescription of up to 2 tablets while 40.0 % agreed to adhere to a 3 tablet prescription,40.0% to daily injection, 88.0% to a quarterly injection and 24.0% to a nasal spray. Only 6.5% of the subjects with diabetes said they would spend as much on osteoporosis as on diabetes while 93.5% were willing to spend less on osteoporosis. The respondents revealed that limitations to accessing osteoporosis care included lack of finance (100.0%), difficulty in travelling (61.8%), lack of support from family members (74.2%), preexisting burden of treatment (74.2%), and the feeling that osteoporosis was not a serious disease (82.6%). This study reveals the importance given to shared decision making by patients with osteoporosis, and the beneficial effect of educational intervention on improving attitude towards therapy in them.

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BACK MUSCLE STRENGTH IS ASSOCIATED WITH BONE MINERAL DENSITY CHANGE IN HEALTHY PREMENOPAUSAL WOMEN: SENDAI BONE HEALTH CONCEPT STUDY

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Aim: The purpose of this study is to examine the association between back muscle strength change and bone mineral density (BMD) change during one-year exercise program. Methods: This analysis was based on a randomized exercise intervention trial in order to evaluate the effects of a high impact exercise on BMD. Ninety-one healthy, non-pregnant premenopausal female employees aged 25-50 at a telephone company participated after full informed consent. Participants were randomly allocated into the following 2 groups; low-impact exercise group (LIE, 46 women, exercise program: Tai-Chi and/or step exercise, 10~15 min/session, 3-5 sessions/week) and high-impact exercise group (HIE, 45 women, exercise program: 5 x10 (max) jumps inserted during LIE; 10~15 min/session; 3-5 sessions/week). Thirty-two LIE and 35 HIE subjects completed a 1-year intervention. BMD in the lumbar spine and proximal femur was measured by dual energy X-ray absorptiometry. Back extensor strength was measured by a transducer (ZPS-DPU-1000N, IMADA, Japan) according to the method published by Limburg PJ(1). Multiple linear regression analysis was used to establish the relationship between back muscle strength change and BMD change after adjustment for age, BMI, physical activity and Ca intake. Results: Mean value (• }SD) of back extensor strength at 12 months increased significantly in each group, from 24.8 (• } 7.2) to 28.6 (• } 9.4) kg in LIE, and from 26.8 (• } 8.6) to 27.8 (• } 8.4) kg in HIE. The multiple regression models showed a positive and significant relationship between back extensor strength change and L3 BMD change during follow-up in LIE and HIE groups, and all subjects combined($p < 0.01$). No relation was observed between back extensor strength change and BMD changes of other sites. Conclusion: Back extensor strength was significantly associated with lumbar spine BMD change after exercises intervention. This result suggests that gaining back extensor strength may be beneficial for lumbar spine BMD. Further study is required to clarify the causality. The contents of the abstract had been submitted and accepted for WCO, P411FR Osteoporosis Int (2008) 19 (Suppl. 2).

(1) Limberg PJ et al. Mayo Clin Proc 66: 39-44, 1991

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LONG-TERM EFFECTS OF EXERCISE AND CALCIUM-VITAMIN D_3 SUPPLEMENTATION ON BIOCHEMICAL MARKERS OF BONE TURNOVER AND THEIR ASSOCIATION WITH CHANGES IN BMD IN OLDER MEN

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Purpose: Bone turnover markers (BTMs) are useful for monitoring the efficacy of antiresorptive therapy on osteoporosis, but few studies have examined the long-term effect of exercise on BTMs in healthy older men. It is also not known whether combining exercise and calcium-vitamin D could lead to a greater reduction in bone markers, or whether BTMs can predict exercise-induced changes in BMD. The aims of this study were to: 1) examine the independent and combined effects of exercise and calcium-vitamin D_3 fortified milk on BTMs in older men, and 2) evaluate whether BTMs were predictive of BMD changes in response to the intervention. Methods : In this 18-month RCT, men (n=180) aged 61.0±7.4 years (±SD) were randomised to resistance training (60-85% 1RM) plus moderate impact exercise with or without calcium-vitamin D_3 (1000mg/d+800IU/d). We assessed FN, hip and LS aBMD (DXA); mid-femur, mid-tibia, distal tibia and LS vBMD (QCT), and serum P1NP and CTx. Results : 172 men (95%) completed the study; exercise attendance averaged 63% and compliance with the fortified milk averaged 90%. There were no exercise-calcium-vitamin D_3 interactions on any BTM or BMD measure, so the main effects of calcium-vitamin D_3 and exercise were analysed. Calcium-vitamin D_3 resulted in net 16% and 14% reduction in serum P1NP ($P<0.001$) and CTx ($P<0.01$), respectively, after 6 months, and these benefits were sustained after 12 months (16-17%) and, to a lesser extent, after 18 months (9-10%). However, these changes in BTMs did not translate into any long-term benefits in BMD. Conversely, exercise resulted in a significant 1.8% and 2.2% gain in FN aBMD ($P<0.001$) and LS trabecular vBMD ($P<0.05$), respectively, but there was no effect of exercise on any BTM. Linear regression analysis also revealed that baseline BTMs were not related to the exercise-induced gains in BMD. Conclusion : A multi-component exercise program in older men enhances BMD at loaded sites, but these benefits were not associated with decreases in BTMs. While bone markers provide an index of total skeletal turnover and are responsive to increases in dietary calcium-vitamin D_3 intake, they appear not be as sensitive to site-specific effects of exercise in older men.

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QUANTIFYING THE VOLUME FRACTION AND TEXTURE OF CANCELLOUS BONE USING 3.0 TESLA MAGNETIC RESONANCE IMAGING

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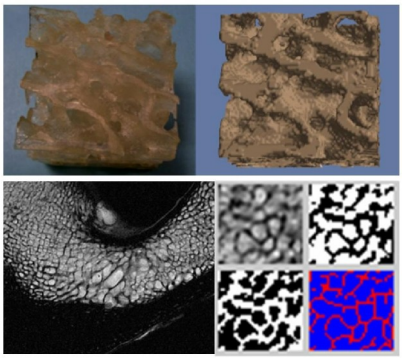
Introduction: 3.0 Tesla MRI offers the potential to quantify the volume fraction and structural texture of cancellous bone, along with quantification of marrow composition, in a single non-invasive examination. This study describes our preliminary investigations to identify parameters which describe cancellous bone structure including the relationships between texture and volume fraction.

Methods: Measurements of bone volume fraction (BVF) and structural texture were performed using a 3.0 Tesla GE Signa MRI system. Software was developed (MATLAB) for segmentation and analysis. MRI scans were performed on four stereolithographic (STL) replica phantoms of natural bone tissue samples (15x spatial magnification) and a porcine calcaneus that had previously been scanned by quantitative computed tomography (QCT).

Results: MRI assessment of BVF correlated significantly for both the STL models ($R^2 = 99\%$) with their known values and for the porcine calcaneus ($R^2 = 95\%$) with QCT. Further, regions of porcine cancellous bone demonstrating a 29% variation in BVF produced variations in textural parameters from 5.5% (homogeneity) to 224% (correlation).



The Figure shows a photograph and 3D MRI image of one STL phantom (top), an MRI image of the porcine specimen (bottom left), the three processing stages utilised to obtain the BVF map, along with structural skeletonisation in red.

Conclusions: We have developed an *in vivo* imaging and post-processing methodology which utilises a clinical high-field MR system and in-house software that permits the detailed analysis of trabecular structure combined with marrow quantification. This will hopefully provide the basis for assessing the multi-factorial nature of bone density, cancellous structure and marrow composition in osteoporosis.

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DEVELOPMENT AND SCIENTIFIC VALIDATION OF A NEONATAL ULTRASOUND AXIAL BONE SCANNER

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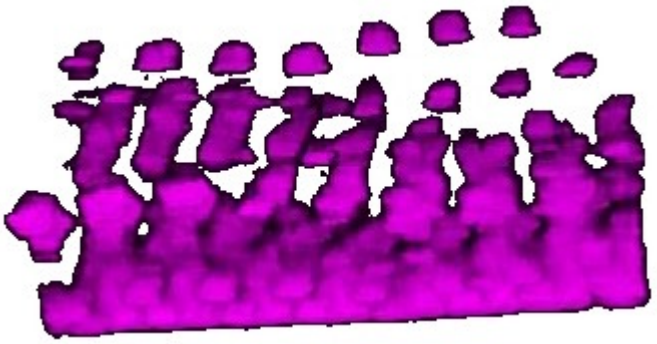
Preterm infants have an increased risk of low bone mass and subsequent fracture due to limited bone mass accretion in utero and a greater need for bone nutrients. The diagnosis of osteopenia of prematurity remains difficult as there is no screening test which is both sensitive and specific. Although dual energy X-ray absorptiometry is increasingly used to assess bone mineral status in newborn infants, it is unsuitable for routine use in the setting of the fragile very low birth weight infant. Ultrasound assessment of bone by ultrasound has to date measured the peripheral skeleton using surface- and through-transmission techniques. This project is developing a neonatal ultrasound axial bone scanner utilising novel multi-frequency 3D pulse-echo technology.

Methods: A twin-compartment water tank, facilitated via an ultrasonically transparent membrane, has been constructed. The neonate will lie in an upper-compartment shallow pool of disposable water, thereby facilitating ultrasound coupling and reducing risk of cross-infection. A one-dimensional motorised scan of a 128-element 2.25 MHz broadband phased-array ultrasound transducer is performed within the lower compartment. Reflected ultrasound signals from the spinal vertebrae are collected, signal processed and analysed, providing both qualitative (3D image) and quantitative (e.g. attenuation) information.

The technique is currently being scientifically validated using a spine phantom derived from a microCT scan of a neonatal piglet, which has been replicated using stereolithography into a solid resin model.

Results: The images show the scanning tank and the first 3D reconstructed ultrasound image of the porcine spine phantom.



Discussion: An ultrasound neonatal scanning system has been developed and 3D images created. The inherent spatial artefact due to differences in tissue velocities is currently being addressed. This will be followed by quantitative assessment of volumetric bone mineral density.

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PREDICTION OF ADJACENT VERTEBRAL FRACTURE RISK ASSOCIATED WITH VERTEBROPLASTY – A COMPUTER-BASED SIMULATION STUDY

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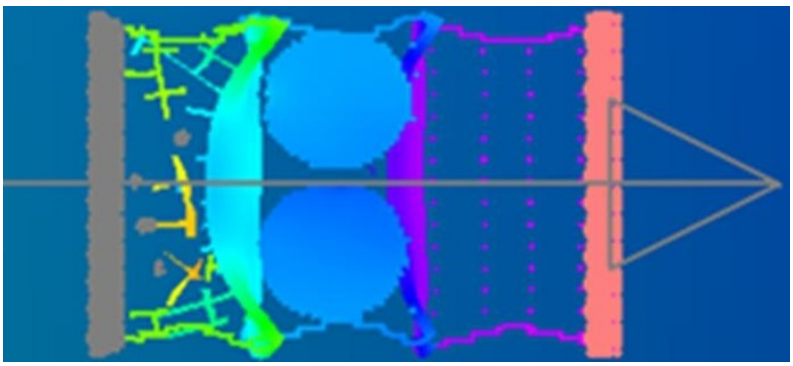
Introduction: Vertebroplasty involves injecting cement into a fractured vertebra to provide stabilisation. There is clinical evidence to suggest however that vertebroplasty may be associated with a higher risk of adjacent vertebral fracture; which may be due to the change in material properties of the post-procedure vertebra modifying the transmission of mechanical stresses to adjacent vertebrae.

Finite element analysis (FEA) is a widely used technique for the computer modelling of structures experiencing a mechanical loading, being inherently sensitive to geometry and material properties, the results expressed as displacement or stress.

Methods: A computer-generated spinal segment model consisting of three vertebral bodies and inter-vertebral discs has been generated. Each vertebral body consists of a convex outer cortex with an internal trabecular lattice structure. Varying degrees of osteoporotic cortical and trabecular degradation of the vertebrae may be implemented. Further, simulated vertebroplasty resin may be incorporated within the central vertebra. Finite element models have been generated utilising Young's modulus values of 18,650 MPa for bone tissue, 75 MPa for inter-vertebral disc, and 2040 MPa for vertebroplasty resin. To date, the primary output parameter has been the mechanical stiffness (N / mm) of the vertebrae.

Results: The stiffness of a vertebra decreased linearly with increased trabecular resorption (-35% stiffness with -38% bone volume fraction), decreased non-linearly with decreased cortical thinning (-58% stiffness with -33% thickness), and increased linearly with increased volume of vertebroplasty resin (+127% stiffness with +500% resin volume). Incorporation of a simulated vertebroplasty increased vertebral stiffness. Simulation of a vertebroplasty into an osteoporotic vertebra positioned between an osteoporotic and a normal vertebra resulted in fracture of the adjacent osteoporotic vertebra when a simulated mechanical loading was applied.



Discussion: This simulation study's initial findings support the hypothesis that the risk of fracture increases with degree of osteoporosis in vertebra adjacent to a vertebroplasty.

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COMPARISON OF 3D FINITE ELEMENT ANALYSIS DERIVED STIFFNESS AND BMD TO DETERMINE THE FAILURE LOAD OF THE EXCISED PROXIMAL FEMUR

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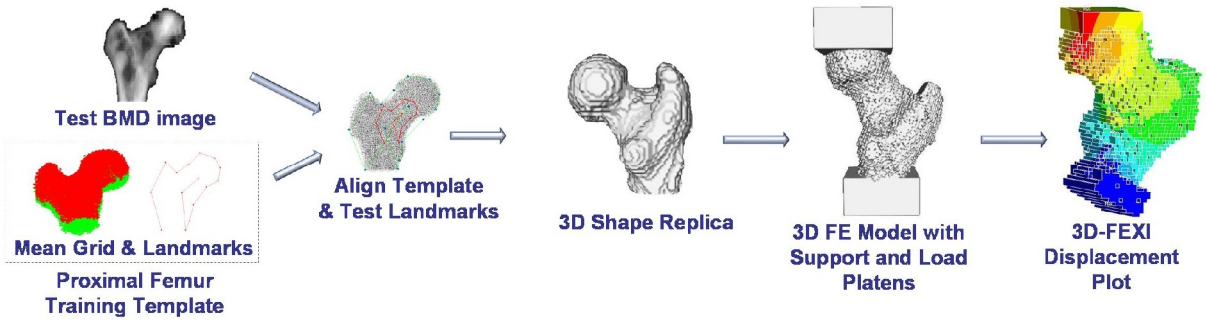
Introduction: Bone mineral density (BMD) is currently the preferred surrogate for bone strength in clinical practice. Finite element analysis (FEA) is a computer simulation technique that can predict the deformation of a structure when a load is applied, providing a measure of stiffness (N mm^{-1}). Finite element analysis of X-ray images (3D-FEXI) is a FEA technique whose analysis is derived from a single 2D radiographic image.

Methods: 18 excised human femora had previously been Quantitative Computed Tomography scanned, from which 2D BMD-equivalent radiographic images were derived, and mechanically tested to failure in a stance loading configuration. A 3D proximal femur shape was generated from each 2D radiographic image and used to construct 3D-FEA models.



Results: The coefficient of determination (R^2%) to predict failure load was 54.5% for BMD and 80.4% for 3D-FEXI. Conclusions: This ex-vivo study demonstrates that 3D-FEXI derived from a conventional 2D radiographic image has the potential to significantly increase the accuracy of failure load assessment of the proximal femur compared with that currently achieved with BMD. This approach may be readily extended to routine clinical BMD images derived by dual energy X-ray absorptiometry.

(1) Langton C M et al; Generation of a 3D proximal femur shape from a single projection 2D radiographic image; Osteoporosis International; In Press

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COMPARISON OF BONE MINERAL DENSITY IN ELDERLY PATIENTS ACCORDING TO PRESENCE OF INTERTROCHANTERIC FRACTURE

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Purpose: To analyze difference in bone mineral density (BMD) between intertrochanteric fracture and control group and to explore predictive value of which BMD for intertrochanteric fracture.

Materials and Methods: 57 patients who were over 60-year-old with intertrochanteric fracture were examined. For control group, 110 patients who did not have any fracture were selected. Dual energy X-ray absorptiometry was studied at 1, 2, 3, 4 lumbar vertebrae, femoral neck, trochanter and Ward's triangle. BMD was compared at each site between two groups statistically.

Results: Fracture group consisted of 16 male, 41 female and was average 70.8 year old. Control group consisted of 21 male, 89 female and was average 68.1 year old. There was no differences in sex and age between two groups ($p>0.05$). BMD of L1, L2 and mean lumbar area were significantly less in fracture group than control group ($p<0.05$). There was no difference between two groups in BMD of another sites($p>0.05$).

Conclusion: BMD of L1, L2 and mean lumbar area in fracture group had lower value significantly, but had no differences between two groups at another sites. BMD of L1, L2 and mean lumbar area might be used as the most sensitive predictive indicator for risk of osteoporotic fractures including intertrochanteric fracture in elderly patient.

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DIFFERENTIAL ATROPHY OF THE LOWER-LIMB MUSCULATURE DURING PROLONGED BED-REST: IMPLICATIONS FOR THE MANAGEMENT OF THE IMMOBILISED PATIENT

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Patients with medical and surgical conditions and also the elderly are often assigned to bed-rest and/or immobilised in orthopaedic devices. Such management approaches lead to musculoskeletal atrophy. Although recovery of bone and muscle after inactivity/disuse is inherently dependent upon resumption of physical activity, little scientific information is available to guide the development of exercise programmes. We sought to quantify and compare the rates of atrophy in the muscles of the lower-limb during extreme physical inactivity (prolonged bed-rest).

10 male subjects underwent 56-days of bed-rest. Magnetic resonance imaging of the lower-limbs was performed at two-weekly intervals during bed-rest. 65 images encompassing the head of femur and lateral malleolus of the ankle (slice thickness=10mm; inter-slice distance=5mm) were collected. One operator measured the cross-sectional area of the following muscles in each image: rectus femoris, vastii, sartorius, gracilis, adductor magnus, adductor longus, biceps femoris long head, biceps femoirs short head, semitendinosus and semimembranosus, gastrocnemius lateralis, gastrocnemius medialis, soleus with flexor hallucis longus, tibialis posterior, flexor digitorum longus, peroneal group, anterior tibial muscles. The volume of each muscle was then calculated and subsequently the relative change compared to baseline. Non-linear mixed-effects models were used to fit an exponential decay model and rates of atrophy were determined.

Rates of atrophy differed ($F= 7.4$, $p<.0001$) between the muscles with the greatest rates of atrophy seen in the medial gastrocnemius, soleus and vastii ($p<.00000002$). The hamstring muscles were also affected ($p<.00015$). Atrophy was less in the ankle dorsiflexors and anteromedial hip muscles. Differential rates of atrophy were seen in synergistic muscles (e.g. adductor magnus>adductor longus, $p=.016$; medial gastrocnemius>lateral gastrocnemius, $p=.002$; vastii>rectus femoris, $p=.0002$). These results demonstrate, for the first time, significantly faster rates of atrophy in the ankle and knee extensors than in other muscle groups and also that synergistic muscles can atrophy at significantly different rates during inactivity. The differential muscle atrophy can lead to the development of muscle imbalances upon return to normal activity. In our opinion, patients subject to bed-rest/immobilisation should be prescribed “closed-chain” resistance exercise, which targets the lower limb antigravity extensor muscles which were most affected in bed-rest.

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RESISTIVE VIBRATION EXERCISE REDUCES LOWER LIMB MUSCLE ATROPHY DURING 56-DAY BED-REST

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Introduction: The development of effective exercise countermeasures to prevent musculoskeletal atrophy during long-term spaceflight is a priority for space agencies around the world. In this study we wish to examine the effectiveness of a resistive vibration exercise countermeasure during prolonged bed-rest in preventing lower limb muscle atrophy.

Method: 20 male subjects underwent 56-days of bed-rest and were randomly assigned to either an inactive control group or a resistive vibration exercise countermeasure group that performed lower-limb exercises (squats, calf raises, toe raises) on a specially designed Galileo Space vibration exercise device (Novotec Medical, Pforzheim, Germany) in 11 exercise sessions per week. Magnetic resonance imaging of the lower-limbs was performed at two-weekly intervals during bed-rest. 65 images encompassing the head of femur and lateral malleolus of the ankle (slice thickness=10mm; inter-slice distance=5mm) were collected. One operator measured the cross-sectional area of the following muscles in each image: rectus femoris, vastii, sartorius, gracilis, adductor magnus, adductor longus, biceps femoris long head, biceps femoirs short head, semitendinosus and semimembranosus, gastrocnemius lateralis, gastrocnemius medialis, soleus with flexor hallucis longus, tibialis posterior, flexor digitorum longus, peroneal group, anterior tibial muscles. The volume of each muscle was then calculated.

Results: Countermeasure intervention reduced or prevented atrophy in medial gastrocnemius, soleus, lateral gastrocnemius and vastii muscles ($F>3.0$, $p\leq.0247$). Atrophy of the peroneals, tibialis posterior, and flexor digitorum longus was also less in the countermeasure group, though statistical evidence for this was weak ($F\leq2.3$, $p\geq.071$). The countermeasure did not prevent atrophy in the hamstring muscles ($F<1.1$, $p>.38$). The adductor longus, sartiorius and rectus femoris muscles showed little loss of muscle volume during bed-rest ($F<2.1$, $p>.088$).

Conclusion: The countermeasure exercise programme was effective in reducing muscle atrophy in the extensors of the knee and ankle. Future work needs to consider optimising exercises to be as time efficient as possible in targeting the muscles most affected in bed-rest/spaceflight.

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LARGE PULMONARY EMBOLUS AFTER PERCUTANEOUS VERTEBROPLASTY IN OSTEOPOROTIC COMPRESSION FRACTURE - A CASE REPORT -

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Percutaneous vertebroplasty for osteoporotic compression fracture or malignant osteolytic spinal tumors provides pain relief. Pulmonary embolism caused by polymethylmethacrylate migration after this procedure is rare and its major complication, pulmonary infarction, involves necrosis of lung parenchyme, resulting from interference with blood supply. We report a case of large pulmonary embolus(diameter 2cm) after cement vertebroplasty for osteoporotic vertebral compression fracture and successful management with anticoagulation only.

INTERNAIONAL SURVEY OF OSTEOPOROSIS IN ASIA (ISOPA)

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Background: Current medical therapy for osteoporosis is evolving due to factors such as development of new drugs, new guidelines, economics of healthcare delivery, and so on. As a result, physicians need to make important decisions related to providing optimal and ongoing therapy for osteoporosis patients.

Aims: In order to explore current practice for treatment plans in osteoporosis patients, a survey of Asian clinicians' views on osteoporosis treatment has been conducted.

Methods: We developed a simple survey for clinicians in nine Asian countries, targeting those doctors that deal with osteoporosis patients in their daily practice. Between 50 and 150 clinicians in each country participated in the study during January and February 2008.Questions included views on combination therapy, medical therapy as a function of disease progression, differences due to sex and age, and so on.Initial responses to the questionnaire were complied during March and April 2008.

Results: Feedback received from a total of 1,034 responders in 9 countries:

China	Indonesia	Malaysia	Myanmar	Singapore	S. Korea	Taiwan	Thailand	Vietnam	Total
151	104	121	131	45	130	131	150	71	1,034

Conclusion: Although data analysis is ongoing, at this time we believe that a large majority of clinicians take deep interest in the issues concerning optimal treatment for each patient at every stage of osteoporosis. However, there is a difference between delivery of ideal therapy and actual practice, due to conditions such as regulation of health insurance, economic situation and so on. Additional findings may be discerned after a final analysis.This preliminary survey may also uncover the need for a more in-depth investigation. The final results and conclusions of the survey may help guide a practical management strategy that will be useful in clinicians' daily practice.

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CALCIUM INTAKE IS NEGATIVELY ASSOCIATED WITH BONE TURNOVER INDEPENDENT OF PTH IN POSTMENOPAUSAL WOMEN

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Aim: There has been evidence that calcium (Ca) deficiency causes bone loss, and Ca supplements have a positive effect on bone mineral density (BMD). Several studies indicated that low Ca intake was associated with increase in bone resorption markers. The aim of this study is to further clarify the effect of Ca intake on bone turnover, and whether the effect is influenced by PTH or BMD.

Subjects and Methods: We enrolled 205 postmenopausal women who had examination of osteoporosis. We measured serum levels of N-terminal propeptide of type I collagen (PINP) and C-terminal cross-linked telopeptide of type I collagen (CTX) by ECLIA, and intact PTH by IRMA, as well as BMD at femoral neck by dual-energy X-ray absorptiometry. Nutrient intakes (protein, fat, Ca, magnesium, phosphorus, sodium, vitamin D and vitamin K) were calculated using dietary records and a food frequency questionnaire.

Results: Mean values of age, body height, body weight, and body mass index (BMI) of the subjects were 63 years old, 152cm, 53kg, 22.9kg/m^2, respectively. Mean daily Ca intake was 655mg, which had almost fulfilled Dietary Reference Intakes for Japanese. Mean BMD value was 0.619g/cm^2 (T score -1.5). Mean serum levels of PINP, CTX and PTH were 54.6ng/ml, 0.404ng/ml and 45.6ng/ml, respectively . Simple regression analysis showed that Ca intake was negatively correlated with PINP ($p<0.0001$) and CTX ($p<0.0001$). PTH had positive correlation with PINP ($p<0.05$) and CTX ($p<0.005$), but not with Ca intake. Ca intake had a weak but significant positive association with BMD ($p<0.05$). Multiple regression analysis adjusted for age, BMI, years after menopause, and all nutrient intakes showed that Ca intake was negatively correlated with PINP and CTX($p<0.01$), but not BMD. Moreover, Ca intake was still significantly correlated with PINP and CTX after additionally adjusted for PTH and BMD as independent variables.

Conclusion: These findings suggest that low Ca intake is the major nutrient factor that could suppress bone turnover independent of PTH or BMD. Increasing Ca intake is important for the prevention of postmenopausal osteoporosis.

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FIVE-YEAR AND 10-YEAR ABSOLUTE RISK OF CLINICAL VERTEBRAL FRACTURE FOR INDIVIDUALS

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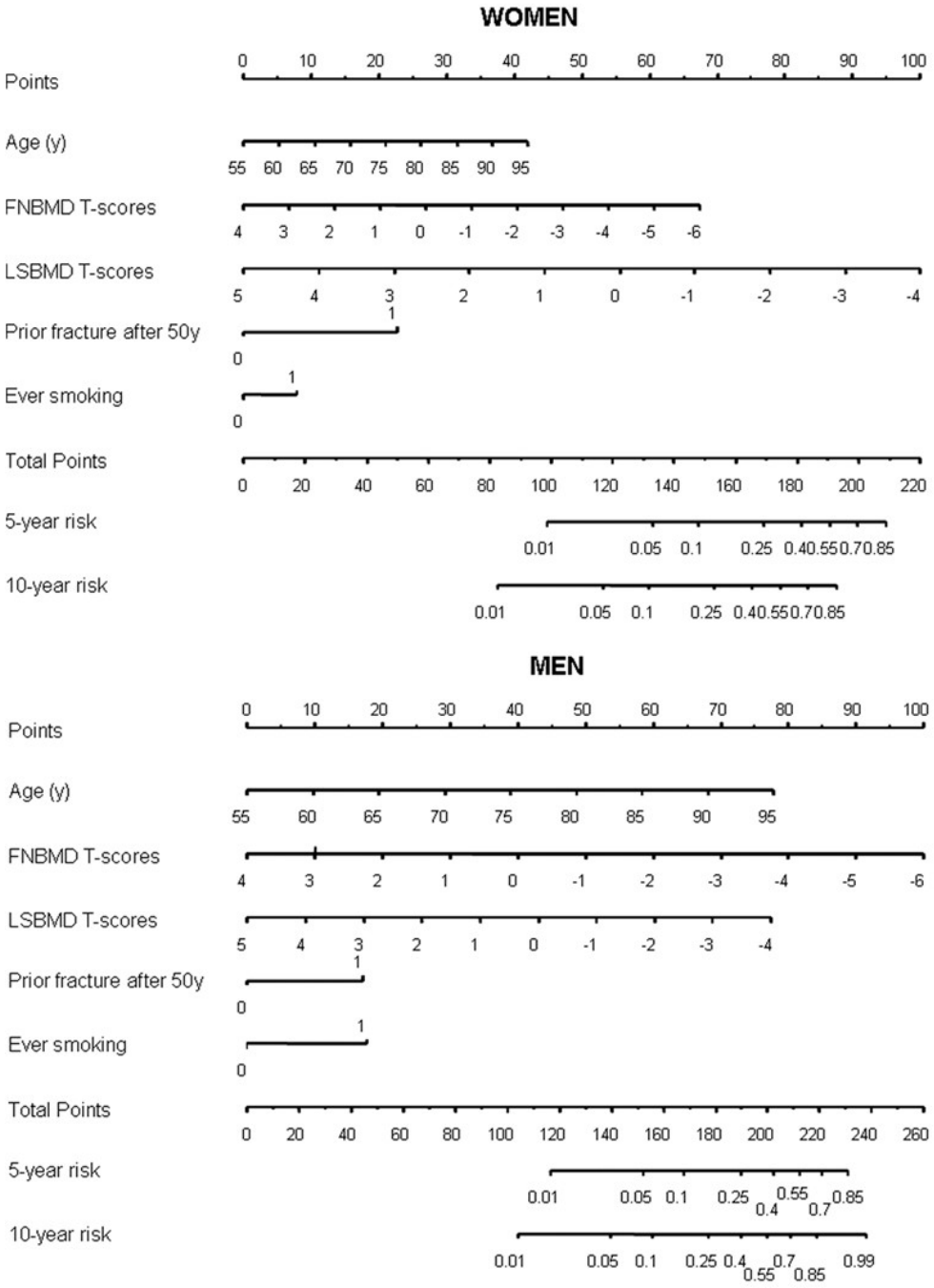
Clinical vertebral fracture is the most common type of osteoporotic fracture in elderly men and women. Because the fracture is associated with increased risk of co-morbidity and mortality, identifying individuals at high-risk for early intervention is a useful approach. The present study was aimed at developing prognostic models to predict 5-year and 10-year absolute risk of fracture for an individual man and woman.

Data from the Dubbo Osteoporosis Epidemiology Study, with 1091 women and 770 men, were analysed. The individuals aged 60+ years as at 1989, who have been followed-up for more than 18 years to ascertain fracture incidence. Baseline measurements included femoral neck (FN) and lumbar spine (LS) bone mineral density (BMD), prior fracture, a history of falls and body weight and other lifestyle factors. Between 1989 and 2007, 159 women and 61 men had sustained a clinical vertebral fracture. Sex-specific best fit models based on the Cox's proportional hazards analysis were chosen, including age, a combination of BMD at the femoral neck and lumbar spine, prior fracture and smoking status for both sexes.

Each 5-y increase in age was associated with a 28% (HR 1.28; 95% CI: 1.13-1.45) increased risk of vertebral fracture in women and 60% increased risk in men (HR 1.56; 1.26-1.93). Femoral neck and lumbar BMD were independent factors for fracture risk (per each SD lower: 1.37; 1.11-1.70 and 1.7; 1.35-2.16, respectively for women and 1.58; 1.18-2.12 and 1.48; 1.08-2.04, respectively for men). The HR for prior fracture was 2.94 (2.02-4.28) for women and 2.18 (1.12-4.24) for men; and the HR for ever smoking was 1.45 (1.03-2.03) for women and 2.24 (1.15-2.43) for men. Based on receiver operating characteristic curve analysis, the area under the curve was 0.75 for women and 0.80 for men. Internal validation of the models showed a good agreement between observed and predicted probability of fracture. Using the models' estimates, various nomograms were constructed for individualizing the risk of fracture for men and women. (Figure)

These data suggest that the combination of BMD at the femoral neck and lumbar spine as well as clinical factors could identify substantially higher individual's risk of fracture. The nomograms presented here can be useful for individualizing the short- and intermediate-term risk of clinical fracture and identifying high-risk individuals for intervention to reduce the burden fracture risk in the general population.



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USING BISPHOSPHONATE AS A PART OF CALCIUM PHOSPHATE CEMENT IN OSTEOPOROTICS PATIENTS FOR LOCAL LOOSENING PREVENTION

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Bisphosphonates are a class of drugs that inhibit osteoclast action and the resoption of bone.

Osteoporosis is caused to abnormal function of osteoclasts. When calcium phosphate cements in osteoprotics patients are used, loosening will be happened because of increased activity of osteoclast in implant situation. If we use bisphosphonates in calcium phosphate cement composition this problem will overcome. In addition as we have investigated the properties of biphosphonate added cement, we observed better properties that only made by some additives, so bisphosphonate could be a very interesting additive too, for achieving to optimum properties.

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EFFECTS OF TREADMILL RUNNING AND SWIMMING EXERCISE ON MUSCLES AND BONES IN RATS WITH IMMOBILIZED HIND LIMBS

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Background: Immobilization causes the joint contractures or atrophy of muscle and bone, and these can be the inhibitors in rehabilitation. This study investigated the effects of treadmill running and swimming exercise on muscles and bones atrophy in immobilized model rats.

Subjects and Methods: Eighteen male rats aged 10 week-old were divided into 3 groups (Cont, TR and SE) randomly. All rats were fixed the left hind limbs with plaster casts at the full extensive position in knee and ankle joints for 5 weeks. After this, the plaster casts were removed from all rats. TR group started running on a treadmill at 10 m/min, for 30 min, on 5 days/week for 4 weeks. SE group started swimming in a pool for 30 min, on 5 days/week, at 30Åé, for 4 weeks. After this experiment, the left (fixed limb) and right (intact limb) soleus muscles (SOL) and tibias of all rats were dissected out. A weight and cross-sectional area of SOL were measured and calculated a ratio of fixed limb to intact limb. A specimen of a proximal tibia was prepared and examined under a microscope. It was evaluated differences in SOL weight and cross-sectional area between fixed and intact limb and among same limbs in Cont, TR, and SE groups. Results: A weight and cross-sectional area of SOL in fixed limbs were significantly lower than those in intact limbs in all groups. A ratio of SOL weight of fixed limb and intact limb in TR rats were significantly higher than that in Cont rats. SOL cross-sectional area and a ratio of SOL cross-sectional area of fixed limb and intact limb in TR group were significantly higher than those in SE and Cont groups. Gaps in fixed limbs in Cont rat were remarkably found in areas of growth plate and primary spongiosa compared with intact limbs in Cont rat or fixed limbs in TR and SE rats.

Conclusions: TR and SE were effective on the improvement of muscles and bone atrophy. These activities gave hind limbs the loading in TR and water resistance in SE.

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DIFFERENTIAL EFFECTS OF CALCIUM SUPPLEMENTS ON BONE FORMATION AND RESORPTION MARKERS IN MEN

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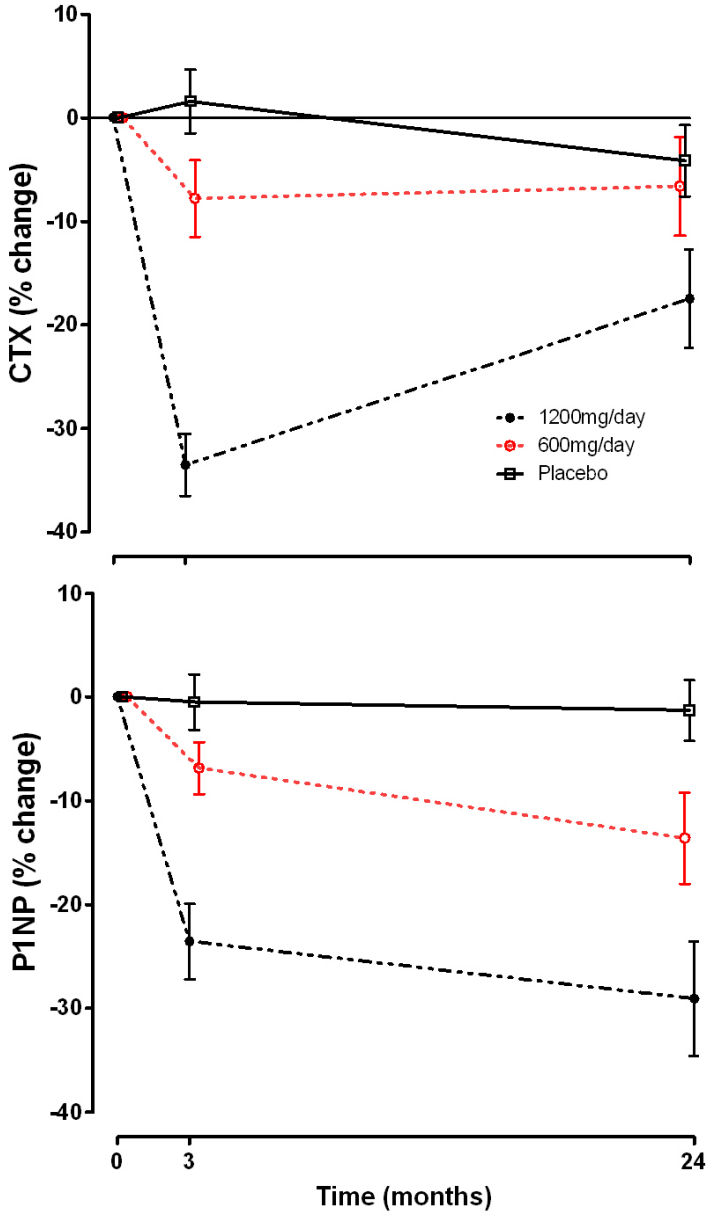
Calcium supplementation is thought to act as an anti-resorptive agent by suppressing PTH, leading to reductions in bone turnover and bone loss. Few studies have assessed calcium supplementation in men. We have recently shown that calcium (as citrate) 600 mg nocte (Ca600) and 600 mg bd (Ca1200), produce dose-related changes in PTH, P1NP, serum alkaline phosphatase and fasting urine calcium excretion. However, Ca600 has no effect on bone density at the spine, hip or total body (Arch Int Med 168:2276). To explain this dissociation of the biochemical and BMD effects of calcium supplementation, we hypothesised that it might have different effects on bone formation and resorption. Therefore, we have now measured fasting serum CTX in these study subjects. Ca600 produced no change in CTX at either 3 or 24 months. Ca1200 decreased bone resorption at both time-points. This contrasts with P1NP which is significantly reduced by both calcium doses. We conclude that calcium 600 mg at night produces a greater long-term suppression of osteoblast activity than it does of osteoclast activity, accounting for its failure to impact on BMD. Therefore, this dose of calcium is unlikely to confer any benefit on bone health in men.



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REGIONAL DIFFERENCES IN THE MANAGEMENT OF OSTEOPOROSIS. THE GLOBAL LONGITUDINAL STUDY OF OSTEOPOROSIS IN WOMEN

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Aim: To compare use of diagnostic technology and osteoporosis medications in women ≥55 years in four geographic regions.

Methods: GLOW is an observational study of women ≥55 years recruited by 615 primary physician practices (17 sites, 10 countries). All non-institutionalized patients visiting the practice within the prior 2 years were eligible. Self-

administered questionnaires were mailed, with 2:1 over-sampling of women ≥65 years. Data collected included demographics; medical history; risk factors for osteoporosis-related fracture; fracture occurrence; self-report of diagnosis and treatment (alendronate, calcitonin, etidronate, ibandronate, pamidronate, raloxifene, risedronate, strontium ranelate, teriparatide, tibolone, zoledronate).

Results: Overall, 70% of the 60,393 women reported having had a bone-density test; frequency ranged from 54% (Europe) to 87% (Canada). Reported use of bone medications (past/current) averaged 27% (range 19% in Europe to 33% in USA). Current use increased with age and clinical risk; 37% of women with FRACTURE Index ≥5 (indicating 26% risk of non-vertebral fracture within 5 years) reported taking bone-related medication. Frequencies ranged from 28% in Europe to 43% in Australia. When use was assessed by self-reported diagnosis of osteoporosis or osteopenia, frequency was 67% and 45%, respectively, with 8.6% of women without either diagnosis reporting use.

Table. Bone-density testing and bone medication by region.

	Bone-density test (%)				Bone medication (%)			
	Australia	Canada	Europe	USA	Australia	Canada	Europe	USA
All women	76	87	54	81	28	30	19	33
Age >65 years	79	87	54	82	34	35	22	39
Age >75 years	76	84	47	79	41	41	24	42
Increased risk of fracture								
-Fracture history	86	89	63	84	49	45	33	47
-FRACTURE Index ≥5	79	85	55	80	43	41	28	42
Self-reported diagnosis (age-standardized to entire population)								
-Normal BMD	63	79	37	69	11	8.8	6.8	10
-Osteoporosis	95	97	87	95	67	66	56	76
-Osteopenia	96	98	85	97	39	46	27	53

Conclusions: Management of osteoporosis is not entirely consistent between Europe and other regions involved in GLOW. Use of bone-density testing and bone medications is lower in Europe. Even in women with high FRACTURE Index scores, Europe appears to be more conservative in using medications.

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AN EVIDENCE BASED APPROACH TO VITAMIN D DEFICIENCY IN THE CHILD AND ADOLESCENT REFUGEE POPULATION OF WESTERN AUSTRALIA (WA)

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Vitamin D deficiency is increasingly common in refugee populations, even in regions with significant sunshine. We investigated (i) the demographic and biochemical predictors of vitamin D deficiency; (ii) the efficacy of different treatment regimens and; (iii) the factors associated with therapeutic outcomes in a retrospective cohort study of child and adolescent refugees resettled in Western Australia (WA).

Between 2005 and 2007, 2000 refugees (median age 18yrs, 48% female) presented for a voluntary initial health assessment shortly after arrival in WA. We included 827 subjects <= 16 years (median age 9yrs, 52% female). 25 hydroxy vitamin D (25OHD, nmol/L) was <27.5 (severely deficient) in 25.5%, <50 (deficient) in 22.1% and <78 (insufficient) in 74.5 %. The prevalence of vitamin D deficiency increased with age (peak: 7.4-13.3 yrs, p=0.01) and in Sudanese patients transiting through Egypt (p<0.001). No biochemical markers were predictive of vitamin D deficiency. Vitamin D3 supplementation was given to 200 individuals (median age 8.9±4.4 yrs, 90 female), of whom 81% were born in Africa. Doses varied between 200-5000 IU of vitamin D3/day for a total of 15-90 days. Preparations included a multi-vitamin suspension, tablets and a concentrate in olive oil (5000 IU/ml). The type of preparation and total dose did not affect the response to therapy, which was universally suboptimal: 25OHD was unchanged in 67.7%, improved in 19.6% and worsened in 12.8%. 59.8% of patients remained vitamin D insufficient (25OHD 27.5-78). Improvements from baseline were more significant in patients <5 yrs of age, if 25OHD was <27.5, in patients from Africa (p=0.002) and if therapy was commenced during winter (p=0.007).

We concluded that vitamin D deficiency and treatment response can be predicted by age, country of transit and season. Current treatment regimens are suboptimal and do not increase vitamin D levels to recommended levels. Doses higher

than 5000 units of vitamin D3/day are likely to be needed. Non compliance, although not directly assessed, may be a contributory factor. Depot oral therapy (stoss-therapy) should be considered as a therapeutic option and prospective randomised trials to establish evidence based therapeutic guidelines and support by public health initiatives are underway.

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PATHWAY TO OSTEOPOROSIS TREATMENT: PATIENT PERSPECTIVES

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The objective of our study is to understand factors that influenced fragility fracture patients' adherence with our hospital's osteoporosis coordinator's recommendations for further osteoporosis investigation, testing and treatment. Research indicates that coordinator-based intervention s are an effective way of initiating post fragility fracture osteoporosis investigation and management. The results also helped identify key variables to consider when evaluating a provincial osteoporosis screening program which is based on the coordinator model.

This was a qualitative study using focus-group methodology. Out of 45 patients who were eligible based on the study criteria, 24 patients participated in five focus groups. Transcripts of the five focus groups were transferred to N-Vivo for storing and sorting the data. By using the qualitative method, we hoped to explore the impact of our hospital's osteoporosis program in a more contextualized fashion.

Our main finding indicates that after making a link between a fragility fracture and osteoporosis, patients must also make an action-oriented appraisal if they are to start osteoporosis treatment. We define two groups of patients: “seekers” and “non-seekers”. For the seekers group, this appraisal was driven by either a fear of another fracture or a desire to maintain a good quality of life. The group of “non-seekers”, who solely depended on what their physician advised, would initiate treatment only if it was physician-recommended. A number of factors influenced each part of our model.

The results of this study fit with the Andersen's behavioral model of health care utilization. Predisposing factors that we identified include personal approach (“seekers” and “non-seekers”), previous knowledge and experience. Need factors were system-based for “non-seekers” but patient-defined for “seekers” who tend to actively seek health information. A number of factors acted as either enablers or barriers within the environmental context of current health care in Ontario.

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EFFECTS OF TREADMILL RUNNING ON BONE MICROARCHITECTURE IN OVARIECTOMIZED RATS

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Background: It is reported that treadmill running inhibits bone loss in ovariectomized (OVX) rats. But the effects are various by the exercise conditions. This study investigated the effect of treadmill running on bone microarchitecture in OVX rats.

Materials and Methods: Twenty-eight female Wister rats aged 8-week- old were divided into 3 groups randomly. One group had Sham-operated (SHAM), and other groups had OVX (OVX, EX). One of OVX groups (EX) started to run on a treadmill at 20 m/min, for 30 min, on 5 days/week, for 8 weeks at a week after the operation. After this experiment, tibias in all rats were dissected out. Metaphysical region of the proximal tibia was scanned with Micro CT, and bone volume (BV) , tissue volume (TV), BV/TV, trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), trabecular spacing (Tb.Spac), stracture model index (SMI), marrow space star volume (V*m space) and trabecular star volume (V*tr) were measured with bone analysis software, TRY/3D-BON. The differences of the bone microartitecture parameters among SHAM, OVX and EX rats were examined.

Results: BV/TV in OVX and EX groups were significantly lower than that in SHAM group, and BV/TV in EX group was significantly higher than that in OVX group. Tb.Th and Tb.N in OVX rats were significantly lower than those of SHAM rats. Tb.Sp and Tb.spac in OVX and EX groups were significantry higher than those in SHAM group. SMI in OVX rats were significantly higher than that of SHAM rats. V*m.space in OVX group was significantly higher than

that in SHAM group. V*tr in OVX and EX groups were significantly lower than that in SHAM group. Conn.D in OVX and EX groups were significantly lower than that in SHAM group.

Conclusion: Treadmill running inhibited bone loss in OVX. Compared with OVX, BV in EX group was kept, and this was considered that the decrease of Tb.Th, Tb.N, Conn.D and increase of Th.Sp, Th,spac were inhibited. Treadmill running was effective on keeping Tb.Th rather than Th.N, especially.

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HIP FRACTURE IN AUCKLAND - CONTRASTING MODELS OF CARE IN TWO MAJOR HOSPITALS

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Background: The optimal setting and design of care for elderly hip fracture patients is unknown. North Shore Hospital (NSH) and Middlemore Hospital (MMH) are two major hospitals in the Auckland region operating different models of orthogeriatric care.

Aim: To compare hip fracture care between NSH and MMH.

Methods: A retrospective case record audit of patients aged 65 and over with hip fracture from July to December 2004 at MMH and NSH.

Results: Charts for 203 patients (101 MMH, 102 NSH) were reviewed. The two groups were similar in age (mean age=83.2 years), gender (80% female), and other casemix factors. Median time from admission to theatre was shorter in NSH, (21 vs. 44 hours, p-value <0.0001). Length of stay was significantly shorter at NSH (mean difference 4.4 days 95% CI 1.1-7.6 when adjusted for casemix factors). Significantly more NSH patients were transferred for rehabilitation than MMH patients (75% vs. 51%). At discharge, significantly more MMH patients (34% vs. 14%) were treated with alendronate. Of 126 patients admitted from home, 81% returned home, 4% went to rest homes, 13% to private hospitals and 2% died; differences between centres were not significant. Overall inpatient mortality was 3.9%.

Conclusions: The orthogeriatric model of care at NSH was associated with a shorter overall length of stay, earlier transfer to the AT&R setting, and a higher proportion rehabilitated in AT&R. Outcomes in terms of discharge destination and six month mortality were similar at both centres.

Key words: Hip fracture, orthogeriatrics, rehabilitation, elderly, osteoporosis

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CAN WE USE NITRIC OXIDE DONOR, NITROGLYCERINE FOR PREVENTION OF POSTMENOPAUSAL BONE LOSS?

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Since medications are expensive, cost-effective therapies are welcome for the management of osteoporosis. The use of HRT declined after a Women's Health-Initiative study that showed increased risks with HRT. The beneficial effects of estrogen on bone maintenance at least in part mediated via nitric oxide (NO)/cGMP pathway. At appropriate doses, nitroglycerine as a NO donor was shown to favorably affect both osteoblasts and osteoclasts (i.e., uncoupling these two cell types).

A three-year randomized, doubled-blind, controlled clinical trial was conducted to assess the efficacy of nitroglycerin in preventing bone loss in early postmenopausal women. This study, Nitroglycerin as an Option: Value in Early Bone Loss (NOVEL), was funded by NIAMS. Over 200,000 women were contacted, 1,400 were interviewed, 215 were screened, and 186 were recruited. Women were randomized to receive either nitroglycerine ointment or placebo ointment. All women received calcium and vitamin D supplementation. There were no differences in the treatment arms in key baseline characteristics including BMD, BMI, smoking status, time since menopause, etc. Taking compliance (~75%) into consideration, the actual dose used by the study participants is only ~50% (14 mg/day) of that was originally intended to be used in this study.

The intent to treat analysis did not reveal differences between the two treatment groups on the primary outcome of lumbar spine BMD. Change of BMD from the baseline in each group was only -0.023 (2.1% change over the three-year study period; was not significant). Except for the increase headaches in the active arm, all other adverse events

had similar profiles compared to placebo. Some of the secondary outcomes including biochemical markers of bone turnover have not been completed.

These results suggest that nitroglycerin dose used in this NOVEL clinical study was not effective. However, the NOVEL study subjects used only ~half the effective dose of nitroglycerin, in preventing bone loss. Taken the narrow therapeutic margin for beneficial effects of nitroglycerin on bone, this is likely to be the main reason for not having any positive outcome. Therefore, we believe that an additional study using correct dose of nitroglycerin is warranted before eliminating NO donor nitroglycerin as a novel, cost-effective therapy for prevention of osteoporosis.

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BONE DENSITOMETRY MEDICARE CLAIMS SUGGEST INADEQUATE TESTING FOR AUSTRALIAN MEN AND RURAL MEN AND WOMEN.

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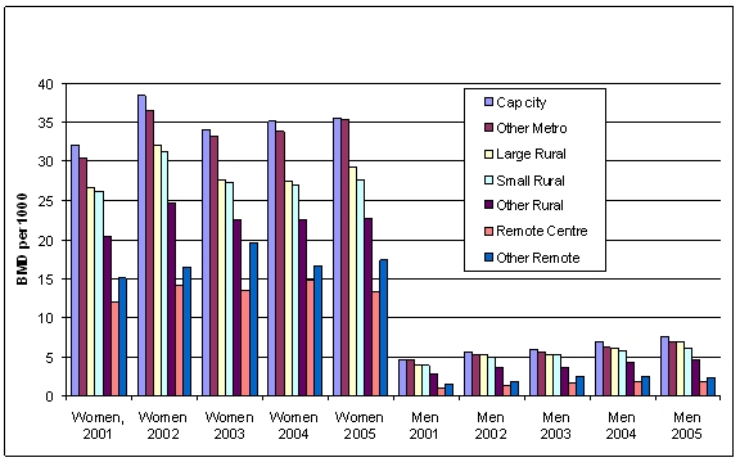
Introduction: There is a substantial gap between evidence-based best practice and actual practice for the detection and treatment of osteoporosis. As part of the preparation of national guidelines for the management of osteoporosis in primary health care, we identified an evidence gap in that equity issues surrounding access to osteoporosis diagnostic and treatment services were under-explored, particularly in the Australian context. Bone densitometry (BMD) utilisation is a key marker of osteoporosis diagnosis and care activity and BMD is the current gold standard for diagnosing osteoporosis. We therefore aimed to analyse sex and geographical differences in BMD utilisation to identify possible access and equity issues in the availability and uptake of BMD.

Methods: We analysed the Medicare claims data for BMD from 2001 to 2005 inclusive. In that time period, Medicare claims covered BMD performed in Australian men and women who had had a minimal trauma fracture, had certain diseases/treatments known to predispose to osteoporosis or who were having BMD to monitor osteoporosis. Age-adjusted BMD rates were derived from Medicare Australia claims and 2001 Australian Bureau of Statistics population data. These were analysed by age, sex and rural, remote and metropolitan areas (RRMA) classification.

Results: Utilisation of BMD increased by 26% over the six years. However rates were lower for rural and remote populations, with people in capital cities about three times as likely to have BMD performed as the remote population (Figure 1). Sex ratios for men remain low, but have improved from >6:1 female to male to 4:1 over the six years.

Discussion : Our results show that there is a relative underutilisation of BMD in males regardless of location, and in rural and remote areas in both sexes. The former is consistent with the known under-diagnosis and under-treatment of osteoporosis in men. The latter likely reflects reduced access to BMD services in these areas. Sex and rural inequities in BMD need to be addressed as part of a national approach to reduce the incidence of minimal trauma fractures and improve ‘second-fracture' prevention.

Figure 1: Age-adjusted BMD utilisation by RRMA location, sex and year



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UNDERCARBOXYLATED OSTEOCALCIN / OSTEOCALCIN RATIO IS NEGATIVELY ASSOCIATED WITH HYPERGLYCEMIC CONDITIONS, BUT NOT WITH THE PRESENCE OF VERTEBRAL FRACTURES, IN PATIENTS WITH TYPE 2 DIABETES.

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Bone fragility of patients with type 2 diabetes (T2DM) depends on bone quality rather than bone mineral density (BMD). A ratio of undercarboxylated osteocalcin level to osteocalcin (ucOC/OC) as well as ucOC level itself is considered as one of candidate index for bone quality. Indeed, ucOC/OC ratio is correlated with sound of speed measured by calcaneal ultrasound, which reflects bone strength independent of BMD. It is well known that OC level is negatively associated with hyperglycemia. However, the associations between bone fragility and ucOC/OC ratio as well as ucOC level in T2DM were still unknown. In this study, to examine this issue, 99 male patients older than 50 years old and 101 postmenopausal female with 2DM within normal creatinine levels were examined by BMD at the lumbar spine, femoral neck and one-third of radius as well as spine radiographs, and by measurement of biochemical parameters including ucOC/OC. Serum level of OC and ucOC as well as ucOC/OC ratio in T2DM women were significantly and negatively associated with HbA1c ($r=-0.24$, $P<0.01$, $r=-0.27$, $P<0.01$, and $r=-0.20$, $P<0.05$, respectively). Serum ucOC level in T2DM men were also significantly and negatively associated with HbA1c ($r=-0.20$, $P<0.05$). Multiple regression analysis was performed with each of OC, ucOC, and ucOC/OC ratio as a dependent variable, and age, body weight, body height, HbA1c, creatinine, BAP, urinary NTX, duration of diabetes, and femoral neck BMD as independent variables. OC, ucOC, and ucOC/OC ratio were negatively correlated with HbA1c in both gender (men: $r = -0.22$, $P < 0.05$, $r = -0.28$, $P < 0.01$ and $r = -0.23$, $P < 0.05$; women: $r = -0.24$, $P < 0.01$, $r = -0.29$, $P < 0.01$ and $r = -0.22$, $P < 0.05$, respectively). These values, however, were not associated with the presence of prevalent vertebral fractures by logistic regression analysis. These results suggest that undercarboxylated OC may not be a predictor of vertebral fractures, although it is decreased by hyperglycemia.

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POSTMENOPAUSAL WOMEN WITH MILD TO MODERATE RENAL DYSFUNCTION ARE AT INCREASED RISK FOR BONE LOSS AND VERTEBRAL FRACTURES

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[Background] An increased rate of hip fractures has been reported in patients with end-stage renal disease, but the effect of less severe renal dysfunction on bone loss and fracture risk is uncertain. We therefore evaluated whether or not mild to moderate renal dysfunction affects bone mineral density (BMD) and the risk of vertebral fractures (VFs) in postmenopausal women. [Methods] We enrolled 659 postmenopausal women who had examination of osteoporosis (mean age 65 years) . Serum creatinine level was measured and creatinine clearance (CCr) was calculated using the Cockcroft-Gault formula . Estimated glomerular filtration rate (eGFR) was calculated using the formula presented by the MDRD study. BMD values of the lumbar (L), femoral neck (FN), and radius (Rad) were measured by DXA. [Results] Renal function was categorized by the criteria from the Kidney Disease Outcomes Quality Initiative (K/DOQI) committee. At baseline, 22.3% of participants had chronic kidney disease (CKD) stage1 (eGFR90 □ ml/min/1.73m^2), 64.8% had CKD stage2 (60–89), and 12.9% had stage3 or more (<60). Based on CCr, these percentages were 46.3%, 46.9%, and 6.8%, respectively. One hundred eighty women had one or more VFs. Comparison of fracture prevalence by CKD stages revealed that the stage3 or more group by eGFR had a significantly higher rate of VFs (45.3%) than the stage1 (23.8%) and 2 (25.3%) groups ($p<0.01$), suggesting that lower eGFR was associated with increasing risk for VFs. In the stage2 group, there was significant positive correlations between eGFR and BMD values at FN and Rad, as well as between CCr and BMD values at all sites. Moreover, postmenopausal women with VFs had lower eGFR ($p<0.05$) and CCr ($p<0.01$) values than those without VFs in stage2. When multivariable logistic regression analysis was performed with the presence of VFs as a dependent variable and CCr levels adjusted for years after menopause, smoking habbit, alcohol intake, as well as BMD as an independent variable. CCr levels were identified as a factor associated with the presence of VFs in postmenopausal women (odds ratio 0.38,

95% confidential interval 0.18-0.79 per SD increase, p < 0.01). [Conclusion] The present results suggest that postmenopausal women with mild to moderate renal dysfunction are at increased risk for bone loss and VFs.

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EFFECT OF BETA-BLOCKER AND THIAZIDE USE ON FRACTURE RISK

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Recent findings of potent centrally-mediated bone anabolic pathways acting in part through the sympathetic nervous system suggests that a possible association between beta-blocker and osteoporosis. The present study sought to assess the effect of beta-blocker and thiazide on fracture risk in the elderly.

Data from 3536 participants (1293 women) aged 50+, who were participants of Dubbo Osteoporosis Epidemiology Study were analyzed. Current use of beta-blocker and thiazide was ascertained by direct interview and verification with medical history. Baseline femoral neck BMD was measured by DXA (GE-Lunar). The incidence of fragility fracture was ascertained between 1989 and 2008. Fractures due to traumatic factors and pathological fractures were excluded from the analysis.

In total, in men, 17.6% used beta-blocker (BB) only, 3.3% with combination of BB and thiazide and 5.2% with thiazide only. Between 1989 and 2008, 237 men and 681 women had sustained a low-trauma fracture. As expected, men and women with a fracture were older and had lower BMD than those without a fracture. Men who used only beta-blocker had a significantly lower risk of fracture than those not on beta-blocker (OR: 0.63; 95% CI: 0.43-0.92). A similar trend was observed in women, but it was not statistically significant (0.96; 0.77-1.21). The use of thiazide was associated with an increased risk of fracture in men (1.68; 1.07-2.63) but not in women (1.06, 0.82-1.32). These associations did not change after adjusting for age, BMD and common risk factors. A combination of the use BB and thiazide was non-significantly associated with lower fracture risk in both sexes (0.94; 0.37-2.47 for men and 0.95; 0.61-1.49 for women).

Thus, these data add to the growing evidence that men on beta-blockers have a lower risk of fracture than those without the medication, and this effect was independent of age and BMD.

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BETA-BLOCKERS AND FRACTURE RISK: A BAYESIAN META-ANALYSIS

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The association between beta-blocker (BB) use and fracture is controversial, due partly to the potential bias from observational studies. The present study sought to systematically combine existing data from previous studies to derive a more reliable conclusion of the association.

A systematic search of the literature revealed 14 potentially relevant studies. Ultimately, data from 8 observational studies with current data were obtained and analysed. The studies included 686,286 men and women. Bayesian random-effects meta-analysis was conducted to estimate the relative risk of fracture associated with BB use.

Beta-blockers use was associated with a reduction of any fracture risk by 15% (OR=0.85, 95% credible interval: 0.75-0.97). The probability that beta-blockers use reduces fracture risk by at least 10% and 20% was 0.84 and 0.15, respectively. Under the assumption that bias over-estimates the true OR by 5%, the effect of beta-blockers use on fracture risk reduction became uncertain, with the probability that beta-blockers use reduces fracture risk by at least 10% was only 0.51.

Thus, this analysis suggests that the use of beta-blockers is associated with reduced fracture risk in women and men, but the association is modest. Given the high proportion of beta-blockers use in the community, the finding may have important implication in the prevention of fracture in the general population .

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NOT ONLY VITAMIN K BUT ALSO BISPHOSPHONATE NORMALIZE THE SERUM UNDERCARBOXYLATED OSTEOCALCIN LEVEL IN OSTEOPOROSIS PATIENTS

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Osteocalcin (OC) is a vitamin K-dependent bone matrix protein, and fully carboxylated OC has a high affinity for hydroxyapatite. Undercarboxylated osteocalcin (ucOC) is a marker of vitamin K deficiency, and an increased ucOC level is a predictor of hip fracture. In this study we evaluated the effect of osteoporosis drugs on serum ucOC levels.

Patients and methods: We measured serum ucOC levels in 70 osteoporosis patients (average age 77.6 years) who had received drug treatment. Thirty-nine patients had been taking vitamin D, 9 had been taking bisphosphonate, 12 had been taking bisphosphonate + vitamin D, and 10 had been taking vitamin D + vitamin K, each for at least 6 months.

Results: The average serum ucOC level was 5.77 ng/ml. The serum ucOC level was over the normal limit (4.49 ng/ml) in 45.7% of the patients. The average serum ucOC level was 7.43ng/ml in the vitamin D group, 3.30 ng/ml in the bisphosphonate group, 3.29 ng/ml in the vitamin D + bisphosphonate group, and 4.52 ng/ml in the vitaminD + vitamin K group. Thus the ucOC level was low in patients who had taken vitamin K or bisphosphonate. The ucOC level tended to be higher when the urinary level of type 1 collagen cross-linked N-teropeptides (NTX) was higher. No significant difference in lumbar BMD was seen between the normal ucOC group and the abnormal ucOC group .

Conclusion: It has been reported that the serum ucOC level is significantly higher in osteoporosis patients than in individuals without osteoporosis. However, the effects of osteoporosis drugs on serum ucOC levels have not been reported, and there is no standard therapy for osteoporosis patients with high serum ucOC levels. Our results indicate that the serum ucOC level is affected by not only vitamin K deficiency but also bone turnover. In conclusion, our findings support the suggestion that not only vitamin K but also bisphosphonate may normalize the serum ucOC level in osteoporosis patients.

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RECOMBINANT HUMAN BMP-2 INCREASES TRABECULAR BONE VOLUME IN THE LUMBAR SPINE OF OSTEOPOROTIC SHEEP

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Aim: Most of the available treatments of osteoporosis are systemic. They do not completely eliminate the incidence of fractures and there may be continued bone loss. rhBMP-2 has been successfully used to promote bone formation. The purpose of this study was to promote localized trabecular bone formation by direct implantation of rhBMP-2 in osteoporotic ovine vertebral bodies.

Methods: Osteoporosis was induced in fifteen mature ewes using ovariectomy, low calcium diet and weekly steroid injection until the lumbar spine bone mineral density (BMD) measured by DXA was reduced by at least 25%. After induction, steroid dose was ceased gradually over one month and rods containing inert carrier alone (control) or rhBMP-2 (0.5 ± 0.08 mg) in either slow or fast release formulation were implanted directly into three adjacent lumbar vertebrae of each animal. There were six control sheep (sham surgery and normal diet) whose spines were harvested after three months. Vertebral BMD was monitored progressively. After three and six months the spines were harvested for micro CT analysis for trabecular bone volume (BV/TV), bone surface, thickness, spacing and number. Changes in BMD and bone morphometric parameters were examined using ANOVA and Tukey's test.

Result: After five months of induction lumbar BMD was reduced over 25% ($p<0.05$), but did not change significantly either immediately after cessation of steroids or for the remainder of the study (to six months). Micro CT data showed increased BV/TV in the vicinity of the rods containing fast release implants after six months (34.6%) compared to the control implants (29.3%, $p<0.05$). The vertebrae implanted with rhBMP-2 showed a 2.9 fold larger area focally devoid of bone in the region of implantation compared to the control implants at three months. However the void size reduced after six months in all treatments.

Conclusion: Increased trabecular bone volume adjacent to the implants suggests that rhBMP-2 could be used for localised augmentation of osteoporosis at a site susceptible to fracture within a short time period (2 months). Further refinement of delivery and dosing regimen may minimize the size of focal voids in the region of the rhBMP-2 implantation.

ONCE-YEARLY TREATMENT WITH ZOLEDRONIC ACID CONTINUES TO BE EFFECTIVE IN OLD AGE

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Background: Bone fragility contributes to 40% of fractures in women aged ≥75 years. A vast majority of elderly patients receive no anti-osteoporotic treatment, the general perception being that it is too late to alter the disease course. Of those treated, ~20-30% patients may abandon treatment within 6-12 months. This analysis was aimed to determine the efficacy of once-yearly i.v. zoledronic acid (ZOL) in reducing the risk of vertebral and non-vertebral fractures in postmenopausal women aged ≥75 years with osteoporosis.

Methods: In this study, data were pooled from two randomized trials, HORIZON Pivotal Fracture Trial and the HORIZON Recurrent Fracture Trial (H-RFT), where women (aged ≥75 years) with documented osteoporosis or a recent hip fracture were randomly assigned to receive an annual i.v. infusion of ZOL 5 mg (n=1,961) or placebo (n=1,926) at baseline, 12 and 24 months. Morphometric vertebral fractures were not assessed in H-RFT, hence the focus of this analysis was on clinical (including clinical vertebral) fractures.

Results: Risk reductions for clinical fractures at 1- and 3-years were statistically significant (p=0.026 and p<0.0001, respectively). Incidence of new clinical fractures over 1 year was 4.12% with ZOL *vs*. 5.74% with placebo [hazard ratio (HR), (95% CI)=0.72, (0.54-0.96)]. Over 3 years, the incidence was 10.79% with ZOL *vs.* 16.64% with placebo [HR, (95% CI)=0.65, (0.54-0.78)]. Subgroup analyses revealed 3-year risk reductions of clinical vertebral [HR, (95% CI)=0.34, (0.21-0.55), p<0.0001)] and non-vertebral fractures [HR, (95% CI)=0.73, (0.60-0.90), p=0.0025)] for ZOL patients.

Conclusion: Once-yearly i.v. infusion of ZOL 5 mg was associated with significant benefits on clinical fractures (including vertebral and non-vertebral) in elderly osteoporotic women. Since non-adherence compromises treatment efficacy with increased medical expenses, an annual infusion of ZOL 5 mg may optimise anti-osteoporotic therapy in elderly patients.

Conflict of interest : Ian Reid has received research support from and acted as a consultant for Merck, Novartis, Amgen and Procter & Gamble.

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EFFECT OF A SINGLE I.V. INFUSION OF ZOLEDRONIC ACID ON BONE TURNOVER MARKERS VERSUS ORAL RISEDRONATE IN PATIENTS WITH GLUCOCORTICOID-INDUCED OSTEOPOROSIS

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Objective: This 1-year, randomized, double-blind, double-dummy study evaluated the effects of a single i.v. infusion of zoledronic acid (ZOL 5 mg) versus daily oral risedronate (RIS 5 mg) on bone turnover markers in patients with glucocorticoid-induced osteoporosis (GIO).

Methods: Randomized patients were further divided into treatment (>3 months glucocorticoid therapy [ZOL, n=272; RIS, n=273]) and prevention subpopulations (≤3 months glucocorticoid therapy [ZOL, n=144; RIS, n=144]). Bone mineral density (BMD) was measured at Months 6 and 12. The change from baseline in serum levels of β-C-terminal telopeptides of type 1 collagen (β-CTx) and procollagen type 1 amino-terminal propeptide (P1NP) were measured on Days 9–11 and at Months 3, 6, and 12.

Results: ZOL increased the lumbar spine (LS) BMD significantly compared with RIS in both the treatment (4.1% vs 2.7%; p=0.0001) and prevention (2.6% vs 0.6%; p<0.0001) subpopulations at Month 12. β-CTx and P1NP levels were also significantly reduced by ZOL as compared with RIS in the treatment (Days 9–11 onwards) and prevention (β-CTx, Day 9-11 onwards; P1NP, Month 3 onwards) subpopulations (p<0.0001). In the subgroups of patients with/without rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or asthma/chronic obstructive pulmonary disease (COPD), reductions in β-CTx for ZOL versus RIS were significant from Days 9–11 onwards in the combined treatment and prevention subpopulations (p≤0.0004 for all). Reductions in P1NP for ZOL versus RIS were significant from Days 9–11 onwards in patients without RA, SLE, and asthma/COPD. In the subgroups with/without concomitant use of proton pump inhibitors (PPIs), selective serotonin inhibitors (SSRIs), or anti-tumor necrosis factor (anti-TNF) at baseline, there was no difference in reductions in β-CTx levels for ZOL versus RIS (p<0.001). P1NP levels were significantly reduced for ZOL versus RIS from Days 9–11 onwards, regardless of PPI (p≤0.0002) or anti-TNF use (p≤0.0001), but was significantly reduced at all timepoints in patients not taking SSRIs (p<0.001).

Conclusion: A single i.v. infusion of ZOL (5 mg) shows a significantly higher bone turnover suppression than daily oral RIS (5 mg) for up to 1 year in different subgroups of patients with GIO.

Conflict of interest: Professor Sambrook reports receiving consulting or advisory board fees from Merck, Sanofi-Aventis, Servier, Novartis; receiving lecture fees from Roche; receiving grant support from the Australian National Health and Medical Research Council.

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STUDY OF RISEDRONATE THERAPY IN OSTEOPOROTIC PATIENTS USING BOTH ELECTRONIC MONITORING OF PATIENT ADHERENCE AND BONE MARKER DATA

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Introduction: Bisphosphonates have been become an important treatment in the management of osteoporosis. However, several clinical studies provided evidence of poor and inadequate use of bisphosphonates. We sought to learn if this

poor usage also prevailed among Japanese patients.. Electronic monitoring using the Medication Event Monitoring System (MEMS) is widely accepted as the most reliable method for compiling drug dosing histories in ambulatory patients, providing an accurate, objective and up-to-date dosing history of each patient. This clinical study is the first in Japan on bisphosphonate that includes reliable compliance data .

Objectives The objective of this study was to survey adherence to risedronate and to assess the impact of physician's reinforcement on the treatment of osteoporotic patients.

Study Design This was a multicenter prospective phase IV study. Clusters were randomized into two groups, reinforcement RE (+) and non-reinforcement RE (-) centers. In the RE (+) group, urinary NTX level of patients was determined every 12 weeks and asked the patients self-report about drug intake and life style. The physician informed the patient of her (his) dosing history data, provided a comment for enhancement of medication compliance, and whether her (his) urinary NTx levels changed for better or worse. The patients in the RE (-) group were not given such an instruction by the physician. The duration of monitoring was 48 weeks, and all patients were prescribed 2.5 mg risedronate daily.

Results Dosing histories were collected and analyzed in 548 patients, 331 in the RE (+) group and 217 in the RE (-) group. We observed that patient adherence to risedronate therapy was considerably higher than reported in studies done elsewhere. At week 48, 82% of the patients still were continuing with treatment. The shortfall in adherence due to poor execution of the dosing regimen was about 10% throughout the monitoring period. In studies in other countries,, persistence with risedronate was in the range 37 to 59% after 48 weeks. The proportion of patients who were still continuing with the the prescribed dosing regimen was not significantly different between the Re+ and Re- groups.

Discussions In this Japanese study, persistence with the risedronate dosing regimen was much higher than seen in such studies in other countries. There was no significant difference between reinforcement and non-reinforcement groups. More detailed analyses will be reported elsewhere in relation to clinical outcome.

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OSTEPAENIA IN A TEENAGE BOY PRESENTING WITH PREVIOUSLY UNDIAGNOSED GUANIDINOACETATE METHYLTRANSFERASE (GAMT) DEFICIENCY AND RESPONSE TO CREATINE SUPPLEMENTATION

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Case Report: A 16 y.o. fully ambulant boy born to consanguineous Indian parents, presented for assessment of a fragility femoral neck fracture sustained against a background of autism and moderately severe intellectual disability. He had a past history of infantile eczema, and epilepsy treated with anticonvulsants from 2 – 10 years of age, with no further seizures following cessation of anticonvulsants. He had a thin body habitus (see table) with long fingers and a high arched palate. He had no speech and negligible social interaction, but physical examination was otherwise unremarkable. Positive investigations revealed an undetectable serum creatinine and a urinary metabolic screen which showed an elevated GUA:Phe of 160 (<36) and a decreased creatinine of 0.3 mmol/l (1.2 – 29.5) consistent with the diagnosis of guanidinoacetate methyltransferase (GAMT) deficiency. He was commenced on oral creatine 5gm three times daily. Despite improvement in physical activity, height and bone density, there was no discernable improvement in his intellectual functioning. Proton and phosphorous brain and leg magnetic resonance spectroscopy (MRS) was performed at baseline and showed an increased inorganic phosphorus peak and decreased phosphocreatine synthesis in brain and decreased creatine concentration in muscle. Following creatine treatment total brain creatine (^{1}H-MRS) and phosphocreatine/ATP ratio (^{31}P-MRS) content increased to 30% and 60% of control values, respectively. Brain GUA returned to normal levels.

TABLE

Clinical Features	Baseline	After treatment
Age (years)	16.25	19.4
Height – cm (%)	169 (25^{th})	186 (90^{th} – 97^{th})

Weight – kg (%)	41 (3rd)	57 (10th)
Lumbar Spine BMD L1 – L4 g/cm2 (z score)	0.673 (-4.7)	0.982 (-1.90)
Total body BMD g/cm2 (z score)	0.846 (-3.43)	1.016 (-2.10)
Serum creatinine (normal 40 – 100 mmol/l)	< 20	58

Discussion: Creatine deficiency syndrome is a rare disorder first described in 1994 characterised by intellectual disability, mutism, autism, dyskinesia and epilepsy as in our patient. Osteopenia observed by us in this condition is consistent with phosphocreatine being a substrate for alkaline phosphatase, to provide inorganic phosphate to form the bone mineral hydroxyapatite. Creatine supplementation in our patient with GAMT deficiency resulted in improved BMD with no further fragility fractures, despite negligible improvement in intellectual function.

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Category 3.

Osteoporosis, Pathophysiology, Genetics and Epidemiology

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DIFFERENTIAL EFFECT OF BISPHOSPHONATE AND SELECTIVE ESTROGEN RECEPTOR MODULATOR ON APATITE CRYSTALLINITY OF TRABUCULAR BONE IN OVARIECTOMIZED RAT OSTEOPOROSIS MODEL

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Current basic theory of treatment for osteoporosis is to inhibit the loss of bone mass with anti-resorptive drugs. Two major anti-resorptive drugs, bisphosphonates (BP) and selective estrogen receptor modulators (SERM), have been shown to reduce the incidence of fracture in postmenopausal woman; however, these reduce the fracture risk more than would be predicted by the increase in bone mineral density (BMD). Therefore, bone qualitative factors are of great interest in assessing fracture risk and therapeutic effect of anti-resorptive drugs recently. In this study, we investigated the change of bone quality after the treatment of BP or SERM using ovariectomized rat model, focusing on bone apatite crystallinity.

Female Sprague-Dawley rats (n=48) were ovariectomized or sham operated, and divided into four groups: Sham (sham-operated + vehicle), Ovx (ovariectomy + vehicle), Ovx-Rlx (ovariectomy + raloxifene), and Ovx-Aln (ovariectomy + alendronate). Dosing was initiated at four weeks after ovariectomy, and animals were killed at 4 and 8 weeks after dosing. Femurs were harvested and divided into two areas: mid-shaft area, which mainly consists of cortical bone, and epiphysis area, which contains abundant trabecular bone. X-ray diffraction analysis of crystallinity and crystallite length, BMD measurement, and micro-CT histomorphometry were preformed.

In the epiphysis area, both crystallinity and BMD significantly decreased in Ovx compared to Sham, and these tended to recover by Rlx and Aln treatment. Especially in Ovx-Aln, crystallinity recovered to the sham level. In the mid-shaft area, both crystallinity and BMD showed no significant difference among all four groups. Regarding the crystallite length, there is no significant difference among all four groups in both mid-shaft and epiphysis areas.

The results of this study showed that crystallinity of trabecular bone, where bone remodeling cycle is faster compared to cortical bone, decreased under the high bone turn-over condition induced by ovariectomy, and it recovered by anti-resorptive therapy. Possible explanation for this is that faster remodeling cycle shortens secondary mineralization period resulting in inhibition of bone apatite crystallites maturation. In conclusion, from the crystallographic point of view, short-term administration of BP in osteoporotic patients might cause a favorable effect on apatite crystallinity.

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LIFETIME LOADING ENHANCES CORTICAL BONE GEOMETRY AND BONE MASS DISTRIBUTION IN OLDER MEN

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Bones should adapt their strength to increased loading, but since the distribution of loading-induced strains within each skeletal site is non-uniform the level of adaptation is likely to be both site- and region-specific. In this study we investigated the effect of lifetime loading history on: 1) cortical vBMD and its distribution in different directions in the bone cross-section; 2) cortical geometry and strength, and 3) axial and appendicular trabecular vBMD. In men (n=282) aged 62±7 years (±SD) QCT was used to assess mid-femur and mid-tibia vBMD, geometry and strength (*I*polar), polar distribution (a measure of bone mass at different directions in the bone cross-section), radial distribution (a measure of bone mass distribution throughout the cortex), muscle CSA, and L1-L3 and distal tibia trabecular vBMD. Sport/leisure activity was assessed by questionnaire to calculate an osteogenic index (OI) during adolescence and adulthood. Subjects were then categorised into a high (H) or low/non impact (L) group according to their OI scores in each period. Three contrasting groups were then formed: H-H, H-L and L-L. In the H-H and H-L compared to L-L group, TotAr, CortAr, and Ipolar were 3.4 to 8.9% and 5.4 to 20.2% greater at the mid-femur and mid-tibia, respectively ($P<0.01$-<0.001). Mid-tibia CortAr and Ipolar were 4.8-6.2% greater in the H-H versus H-L group ($P<0.05$-<0.01). These results remained unchanged after adjusting for age, bone length or muscle CSA. There was no effect of loading history on cortical or trabecular vBMD or cortical bone mass distribution from the subcortical to periosteal surface at any site. For polar bone mass distribution there were region-specific effects of loading on cortical bone at the mid-tibia and mid-femur which were consistent with the expected patterns of loading-induced strains at these sites (Figure). In

conclusion, in older men a history of participation in weight-bearing activities during adolescence enhanced cortical bone structure and strength, and these benefits were maintained even if loading was reduced during mid-adulthood. In contrast, there was no effect of loading on whole bone cortical (or trabecular) vBMD, but there were regional variations in the adaptation of cortical bone consistent with the patterns of loading.

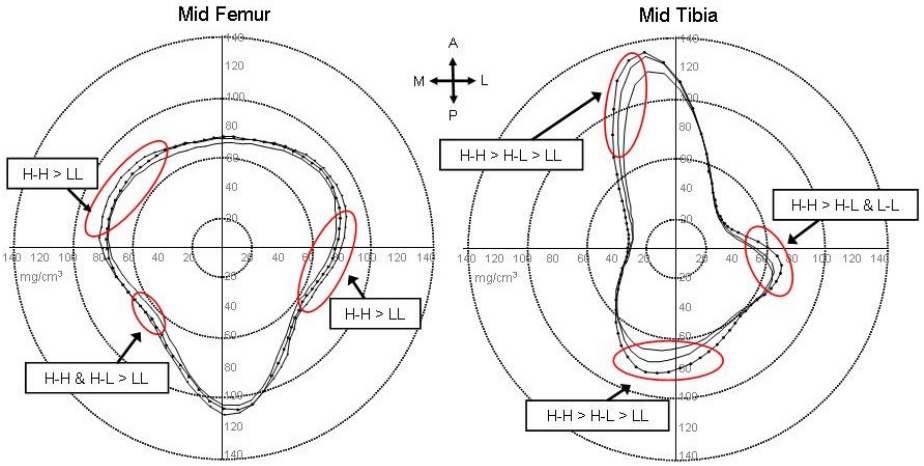

Figure: Effects of lifetime loading history on mid-femur and mid-tibia bone mass distribution (all differences p<0.05-<0.001).

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DAILY EXERCISE IS MOST EFFECTIVE FOR INCREASING HIP BONE MINERAL DENSITY: A RANDOMIZED HIGH-IMPACT, UNILATERAL INTERVENTION.

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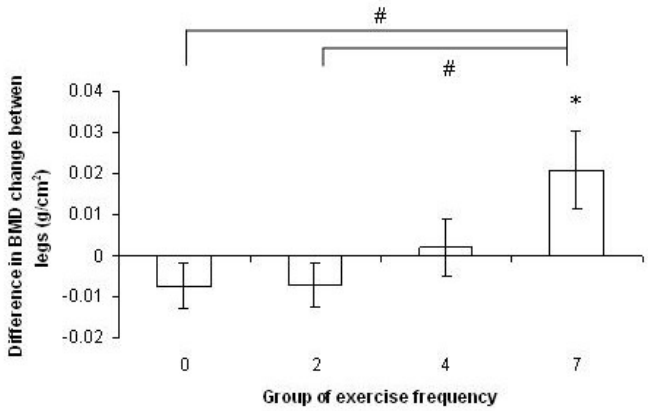
PURPOSE: Brief, high-intensity, and unusually distributed loading may be most effective to increase BMD, but the optimal frequency of such exercise is unknown. Regular bouts of jumping can significantly increase femoral neck BMD[1,2]. Unilateral jumping allows a within subject control limb, thus minimising effects of many confounding factors. This study aimed to compare the effectiveness of different weekly frequencies of exercise using a high-impact, unilateral intervention.

METHODS: Participants were healthy, sedentary, regularly menstruating premenopausal women. They were randomly allocated to perform exercise (incorporating 50 multidirectional hops) on 0, 2, 4, or 7 d/week for 6 months. Exercisers were randomly assigned to exercise on either their left or right leg for the duration of the study. BMD at the lumbar spine, femoral neck, trochanter, and total hip were measured by DXA (Lunar Prodigy Advance) at 0 and 6 months.

RESULTS: 62 women (age 33.6±11.1 years, weight 60.3±9.2 kg, height 1.63±0.06 m) completed the intervention. Compliance amongst exercisers was 86.7±10.6%. The difference in response between legs at the femoral neck was significantly greater in those exercising 7 d/week than in those exercising 0 or 2 d/week (Figure). There were no significant differences in response between limbs in those exercising 0, 2, or 4 d/week. BMD at other sites did not change significantly.

CONCLUSION: Brief hopping exercises performed daily for 6 months increased hip BMD in healthy premenopausal women. Frequent exercise may therefore be most effective for optimising bone health in this population.



*Significant difference in response between legs (p<0.05)

#Significantly different response between legs between groups (p<0.05)

FIGURE: Differences in femoral neck BMD between the trained and control limb in response to a 6-month unilateral exercise intervention, mean (SE).

Disclaimer: The contents of this abstract had been submitted and accepted for the IOF World Congress on Osteoporosis, Bangkok [Osteoporosis Int (2008) 19 (Suppl. 2)].

(1) Bassey EJ and Ramsdale SJ (1998) Pre- and postmenopausal women have different bone mineral density responses to the same high-impact exercise. J Bone Miner Res. 13(12):1805-1813.

(2) Kato T, Terashima T, Yamashita T, Hatanaka Y, Honda A, Umemura Y (2006) Effect of low-repetition jump training on bone mineral density in young women. J Appl Physiol. 100(3):839-843.

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INCREASE IN LUMBAR SPINE BONE DENSITY DURING BED-REST AND RELATIONSHIP TO PARASPINAL MUSCLE ATROPHY

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Purpose: To examine the effect of prolonged bed-rest on spinal bone density (via quantitative computed tomography; QCT) and the relationship to atrophy of individual spinal muscles (as measured with magnetic resonance imaging; MRI).

Method: 9 healthy men underwent 60-days of 6° head-down tilt bed-rest (BR) as part of the 2nd Berlin BedRest Study. The subjects performed no exercise. QCT and MRI were conducted at the 1st, 2nd and 3rd lumbar vertebral levels prior to bed-rest, mid-BR (MRI: days 27 or 28; QCT: day 30) and end-BR (MRI: days 55 or 56; QCT: 6 days after end of bed-rest). At each vertebral level, trabecular bone density was evaluated via QCT and muscle cross-sectional area (CSA) of the lumbar multifidus (MF), erector spinae (ES), psoas (PS) and quadratus lumborum (QL) via MRI.

Results: Significant changes in trabecular (F=3.76, p=.0460) bone density were seen. Trabecular bone density increased +2.8(0.9)%, (p=.0023) at mid bed-rest and +6.8(0.9)%, (p<.00001) by end of bed-rest. Muscle CSA altered significantly (F=20.3, p<.0001) and changes varied significantly between muscles (F=59.2, p<.0001). ES showed the greatest losses in CSA (mid-BR: -8.4(1.5)%, p<.00001; end-BR: -11.4(1.6)%, p<.00001), followed by QL (mid-BR: -3.6(3.)%, p=.24, end-BR: -8.3(1.9)%, p=.00004) and MF (mid-BR: -2.9(2.0)%, p=.16; end-BR: -5.1(1.9)%, p=.011). Surprisingly, PS muscle CSA increased (mid-BR: +5.4(2.8)%, p=.054; end-BR: +8.4(2.5)%, p=.0013). CSA of all muscles combined decreased 10.4(1.6)% (p<.00001) by end-BR. No strong correlations (Spearman's ρ) were seen between bone density changes and muscle CSA changes (p all ≥.049), but younger and lighter (at baseline) subjects exhibited greater increases of bone density during bed-rest (ρ≤-.427, p≤.001).

Discussion: The findings of increased lumbar bone density during bed-rest are in stark contrast to our understanding of the effect of bed-rest on load-bearing bone and also findings in astronauts. The postural muscles of the spine atrophied strongly but the increased size of the psoas muscle, though puzzling, is similar to prior findings. The poor relationship

between muscle-size and bone changes during bed-rest indicates that other, perhaps functional but also non-mechanical (e.g. bone marrow changes), factors need to be investigated to understand the increases in lumbar bone density.

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BONE GEOMETRY AND SURFACES DETERMINE DIFFERENCES IN STRUCTURAL DECAY PRODUCED BY AGE AND MENOPAUSE: A CO-TWIN CONTROL STUDY

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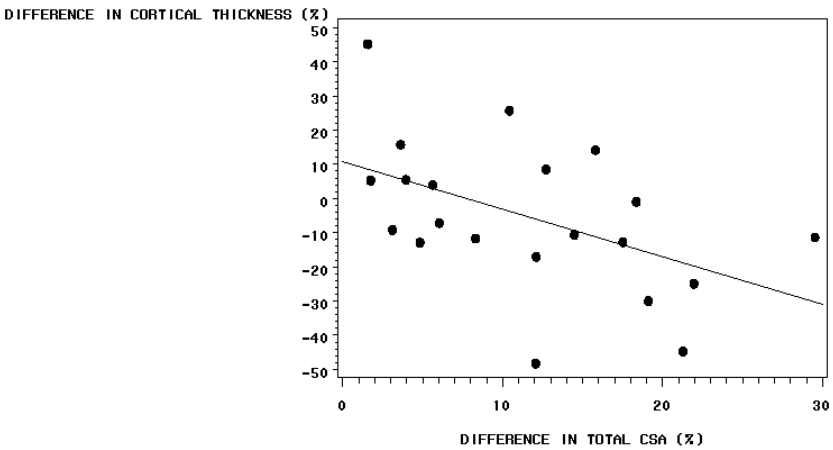
The amount of bone lost after menopause is determined by the magnitude of the negative bone balance between the volumes of bone resorbed and formed by each basic multicellular unit (BMU), the number of BMUs (remodelling rate) and the amount of bone available to be lost. However, remodelling is surface dependent; the amount of surface available determines the accessibility of the bone volume to being remodelled. This is why trabecular bone is lost more rapidly than cortical bone. Larger bones are 'more empty'; they are assembled with less bone within their external (periosteal) envelope to maintain lightness for mobility. We hypothesized that volumetric bone mineral density (vBMD) is lower in women with larger bones because they have more surface for remodelling. We conducted a cross-sectional study of bone structure by high resolution 3-dimensional peripheral computed tomography (HR-3D-pQCT) at the distal radius and tibia in 42 MZ and 21 DZ twin pairs 40-60 years of age. In both the pre- (n = 68) and the postmenopausal group (n = 29) who were not using hormone therapy, total cross sectional area (TCSA) correlated inversely with cortical thickness and with cortical and total vBMD (r = 0.44-0.89, P < 0.001). In postmenopausal women, trabecular vBMD, number and thickness were lower at both sites while cortical vBMD and thickness was lower at the distal tibia, not radius. A 10 year higher age, was associated with 0.2 mm lower cortical thickness in women with bigger tibial bones (TCSA > median) and 0.1 mm lower cortical thickness in women with smaller bones (TCSA < median). We plotted the within-pair differences (% of pair mean) in tibial traits vs. differences (% of pair mean) in totalCSA (bone size); a 10% larger bone was associated with 7.3% lower total vBMD, 13.9% thinner cortex, 4.5% thinner trabeculae and 6.6% higher trabecular number in 21 DZ pairs (Fig), and 7.0% lower total vBMD, 4.5% thinner cortex, 3.9% thinner trabeculae and 2.0% lower trabecular number in 42 MZ pairs. All women become postmenopausal but differences in structural decay are partly the result of bone geometry itself which determines rates of bone loss by influencing the accessibility of bone to being remodelled.

Fig. Differences in Cortical Thickness (%) by differences in Tibial Total CSA (%) in 21 DZ pairs.



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BIOMECHANICAL PERFORMANCE PRINCIPAL COMPONENT LINKAGE MAPPING IN RECOMBINANT CONGENIC MICE: *OSTF* IS A CANDIDATE GENE

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Skeletal fragility is an important health problem with a substantial genetic component. We exploited the genetic structure of recombinant congenic mouse strains by performing a reciprocal intercross of the strains HcB-8 and HcB-23, yielding 603 F2 progeny. The cross was phenotyped for femoral biomechanical performance and geometry. We previously reported the results of linkage mapping for the raw phenotypes. Here, we report principal component (PC) analysis and linkage mapping of the PCs.

We performed PC analysis of 17 raw phenotypes with the prcomp function of R, following normalizing transformations as necessary. Genotyping included 41 loci spanning the segregating regions of HcB-8 and HcB-23. We analyzed the cross with R/qtl and QTL Cartographer and established significance levels by permutation testing.

Four PCs had Eigenvalues > 1, and these accounted for nearly 80% of the phenotypic variance. Inspection of the PC weightings suggest that PC1 is "stiffness-like," PC 2 is "ductility-like," and PC3 and PC4 are "strain-like." Linkage mapping of the entire F2 progeny revealed significant QTLs (with max LOD scores) on chromosomes 1 (7.5), 2 (3.7), 4 (12.4), 10 (2.9), and 19 (4.7). Significant sex x QTL and cross direction x QTL interactions were present, and resulted in additional significant linkages in subgroups of the F2.

Recombination during the generation of the HcB strains confines the possible locations of the chromosome 19 QTL to approximately 10 Mb. Furthermore, the chromosome 19 QTL for PC2 was not found for either the raw phenotypes or the calculated tissue-level mechanical properties. The gene encoding osteoclast stimulating factor 1, *Ostf1*, lies within the chromosome 19 segregating region and is therefore a candidate gene for biomechanical performance. Both PC analysis and use of recombinant congenic strains were important study design elements. PCs provided robust traits for linkage mapping, while the recombinant congenic strains limited the segregating region on chromosome 19 to a short segment.

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PREVALENCE AND TREATMENT OF OSTEOPOROSIS IN OLDER AUSTRALIAN MEN – FINDINGS FROM THE CHAMP STUDY

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Background: In Australia, men are entitled to Government subsided osteoporosis treatment with bisphosphonates if they have: a) previous minimal trauma fracture, b) > 1 vertebral deformity, c) age ≥ 70 with a BMD T-score of -3 or below. It is unclear how many men meet these criteria and whether they are being treated.

Aims: To determine the proportion of older men in the Australian population who meet criteria for osteoporosis treatment and to determine the proportion on effective treatment.

Methods: In 2005-2007, men aged ≥ 70 were recruited for a large epidemiological study focusing on the health of older men : The Concord Health and Aging in Men Project (CHAMP). All men from a defined geographical region in Sydney were invited to participate. Data were collected through questionnaires and clinical assessments. Subjects were asked about previous fractures and falls. Bone mineral density (BMD) of the neck of femur and spine was measured by DEXA scan (Hologic Discovery – W). Vertebral deformities were identified from DEXA vertebral assessment images.

Results: Of the 1705 men seen at baseline, 1662 completed all DEXA scans and 412 (24.7%) met one or more of the criteria for osteoporosis treatment. One-hundred and five men (6.3%) reported having had a minimal trauma fracture in the past 10 years. Of the remaining men, 288 (17.3%) had ≥ 1 vertebral deformity on DEXA imaging. Forty one (4%) men with neither a history of fracture nor a vertebral deformity had at least one BMD T-score of ≤ -3. Few men with osteoporosis were on bisphosphonate treatment. Only 36 (8.9%) of men who met the PBS criteria reported using bisphosphonates, 10 (2.4%) a combination of calcium and bisphosphonates, 48 (11.7%) calcium and 31(7.5%) Vitamin D. Of men who met criteria for osteoporosis treatment, 48 (12.0%), reported ≥ 2 falls during 12 months of follow-up of whom only 3 reported taking bisphosphonates.

Conclusion: In a representative sample of community living older men, approximately 25% met criteria for specific osteoporosis treatment. This was mainly based on pre-existing vertebral deformities. Osteoporosis in older men is under diagnosed and under treated in Australia

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THE ASSOCIATION BETWEEN URBAN OR RURAL LOCALITY AND OSTEOPOROTIC HIP FRACTURE IN COMMUNITY-BASED ADULTS: A SYSTEMATIC REVIEW

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Urban or rural locality has been suggested to influence musculoskeletal health, with lower bone mineral density and greater prevalence of rheumatic diseases identified in urban residents. The relationship between risk of osteoporotic fracture and living in an urban or rural region has been examined in a number of countries, and speculation is wide regarding why differences in bone health are observed. We systematically reviewed the literature regarding urban or rural locality as a risk factor for an increased risk of osteoporotic hip fracture.

A computer-aided search of Medline, EMBASE, CINAHL and PsychINFO, from January 1966 to November 2007, was conducted to identify studies investigating the relationship between urban or rural locality and the occurrence of osteoporotic hip fracture. The methodological quality of studies was assessed using a previously applied scoring system. Due to much heterogeneity, results could not be combined in a meta-analysis, however a best-evidence synthesis of the literature was performed.

Twelve cohort studies and one case-control study fulfilled the predetermined criteria for inclusion. Four studies were of high methodological quality, all of which were cohort studies. This review found strong evidence that residents of rural regions are less likely to experience hip fracture compared to urban residents. Causal factors for fracture rates may include person-level factors such as occupation or physical activity, or area-level characteristics including environmental hazards or climatic extremes.

This systematic review did not identify a definitive reason for lower fracture incidence in rural areas; however further understanding of the relationship between osteoporotic hip fracture and urban or rural locality is required in order to inform potential interventions for fracture risk reduction, and to identify modifiable variables in high-risk populations.

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THE RELATIONSHIP OF POLYMORPHISM OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA GENE(PPARΓ) AND OSTEOPOROSIS IN AGED MALE

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Objective: To study the relationship of the single nucleotide polymorphisms (SNP) of exon6 C161 → T of peroxisome proliferators activated receptor γ (PPAR γ) gene and osteoporosis in Chinese aged male.

Methods: Genomes DNA were extracted from peripheral white blood cells and PCR-RFLP was used to analyze the gene frequency distribution in non osteoporosis (NOP)group and osteoporosis (OP)group in old male. Bone mineral density of lumbar and the upper thighbone (triangle of big rotor, neck of thighbone and Ward ' s) were measured by dual energy X-ray absorptiometry. Serum osteocalcin were measured by ELISA. Results The exon6 of PPAR γ have three genotype(CC,CT and TT).The distribution frequency of genotype accord with Hardy-Weinberg law. The gene frequency of T allete in osteoporosis were higher than that in non osteoporosis . Compare with the control group, OP have a lower level of serum bone glaprotein and bone mineral density. The bone mineral density in the genotype of CT and TT were lower than that in the genotype of CC. Conclusion Our study shows the SNP of 6th exons of PPAR γ may relate to osteoporosis in old males. T allele of PPAR γ may be a impressionable factor of osteoporosis in old males. PPAR γ may be a candidate gene of osteoporosis in old males.

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MECHANISMS OF BONE LOSS IN TWINS DISCORDANT FOR CIGARETTE SMOKING

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Although smoking is recognised as an independent lifestyle determinant of bone mineral density (BMD) and fracture risk, the responsible mechanisms are unclear.

We conducted a cross-sectional study on twin pairs discordant for cigarette smoking seeking mechanisms of smoking - associated BMD deficits. We used the co-twin difference method of analysis on 69 volunteer pairs (13 male and 56 female) aged 40-76 (mean ± SD 53 ± 8.9) years. DXA was used to measure BMD, lean and fat mass ; height , weight and lifestyle factors, including smoking history, menopausal status, exercise and dietary intake were recorded . Blood and urine samples were taken to measure bone biochemical markers and hormones. Percentage w ithin-pair difference (WPD) results shown are for [(smoking twin - non-smoking twin) / mean] x 100.

WPD were seen (95% confidence interval, p Value): Lumbar Spine (LS) -3.5% (-7.0 to -0.0, p=.058), Femoral Neck (FN) -5.6% (-9.0 to -2.2, p=.002), Total Hip (TH) -6.2% (-9.4 to -2.9, p<.000), Forearm (FA) -0.8% (-2.6 to 1.0, p=.290), Whole body BMC (BMC) -4.1% (-7.2 to -1.1, p=.012). Fat mass was also lower in smoking twins, -12.8% (-20.7 to -4.8, p=.005), and lean mass marginally so -2.8% (-5.9 to 0.3, p=.083). Findings persisted after adjusting for age, height, and further adjustment with weight (except FA). Previous research indicated different mechanisms according to gender and menopausal status, thus these subgroups were studied. WPD in subgroups remained similar to group results, with largest WPD seen in post-menopausal women. We found significant WPD in serum 25 OHD (42.8 v 73.3 nmol/L; -46.8%, p=0.02) in pre-menopausal women and this WPD strongly correlated (r=-.47 to -.68, p<0.05) with LS, FN, TH and Whole Body WPD. Post-menopausal women did not show significant differences in calcium or indices of bone metabolism, so BMD deficits in smokers may be due to WPD in Leptin (-18.2%, p=0.04) and fat mass (-11.3, p=0.05). Males showed no significant differences in biochemical markers or hormones.

The findings provide new and important insights into smoking-associated bone deficits. They suggest differing mechanisms in men and women and that relative deficiency of vitamin D may play an important deleterious role in female smokers.

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TWO YEAR RESULTS IN A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF ODANACATIB (MK-822) IN POSTMENOPAUSAL WOMEN WITH LOW BONE MINERAL DENSITY

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Aim: A randomized, double-blind, 2-year study (1 yr dose-ranging + 1-year extension) was performed in postmenopausal women with low bone mineral density (BMD) to evaluate the safety and efficacy vs. placebo of 3, 10, 25 or 50 mg weekly of odanacatib, a selective cathepsin K inhibitor, on BMD, bone turnover indices and histomorphometry.

Methods: Postmenopausal women (N=399, mean age: 64.2 ± 7.8 years) with BMD T-scores ≤-2.0 at the lumbar spine, total hip, femoral neck or hip trochanter and ≥-3.5 at all sites were randomized to receive placebo or 1 of 4 doses of odanacatib. 280 of 320 women, who continued into the 1-year extension, completed 2 years of treatment. Participants and investigators remained blinded to treatment allocation during the extension period with the primary endpoint being

% change vs. baseline in lumbar spine BMD. Trabecular bone turnover was assessed via transilial biopsies obtained from all consenting participants at 24 months.

Results: There were progressive dose-related increases in BMD vs. baseline. Lumbar spine and total hip BMD increased 5.5% and 3.2%, respectively with the highest dose, but were essentially unchanged with placebo (-0.2% and -0.9%). Urinary N-telopeptides (NTx/Cr) and bone-specific alkaline phosphatase (BSAP) decreased 52% and 13%, respectively, with the 50-mg dose, whereas uNTx/Cr decreased 5% and BSAP increased 3% with placebo. Preliminary transilial biopsies (N=27) indicate no significant effect on bone remodeling at the 24-year biopsy time point (table).

Conclusions: Two years of odanacatib treatment in postmenopausal women with low BMD increased lumbar spine and total hip BMD with no evidence of skeletal toxicity.

		Placebo	Odanacatib			
Variable	units	N=6	3mg N=7	10mg N=5	25mg N=6	50mg N=4
Bone Formation Rate (surface) x 100	$\mu m^3/\mu m^2/d$	3.7±1.1	4.9±1.0	1.7±0.5	2.7±0.6	3.3±1.4*
Activation Frequency	/yr	0.50±0.16	0.66±0.15	0.24±0.07	0.34±0.07	0.42±0.17*
Osteoclast Surface/ Bone Surface	%	0.62±0.10	0.58±0.15	0.43±0.16	0.59±0.10	0.61±0.20
Mineralizing /Osteoid surface	%	63±18	82±22	54±11	63±11	43±25
Mineralization Lag Time	days	27±8	178±3	19±5	15±2	32±12
All values are mean ± standard error; *N=3 for these endpoints in this group						

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EXCESS OF POST-FRACTURE MORTALITY AMONG MEN AND WOMEN: A RELATIVE SURVIVAL ANALYSIS. .

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The post-fracture mortality is assumed to be due to fracture and non-fracture causes. Deaths due to"other causes" are reflected by the background mortality rate. The aims of this study were therefore to estimate the "relative survival" among men and women following a fragility fracture, and to estimate the excess of mortality associated with a specific fracture.

The Dubbo Osteoporosis Epidemiological Study was designed as a prospective epidemiologic investigation in which men and women aged 60+ as of 1989 had been followed for 19 years (between 1989 and 2007). During the follow-up period, the incidence of atraumatic fractures ascertained by X-ray reports, and mortality was ascertained by personal report and confirmed by the New South Wales Birth, Death and Marriage Registry. Relative survival ratio (RSR) was estimated by taking into account the age-and-sex specific expected survival in the general Australian population from 1900 to 2007.

During the follow-up period 585 women and 230 men had sustained an incident fracture; among whom, 198 (34%) women and 108 (47%) men died after the fracture. The one-year relative survival after a fracture was reduced by 7% (RSR 0.93; 95% CI 0.88–0.98) in men and 1% (95% CI 0.97–1.01) in women. After 5 years, the relative survival was reduced by 17% (RSR 0.83; 95% CI 0.71–0.96) and 2% in women (RSR 0.98; 95% CI 0.94–1.04). In comparison to forearm fracture, a hip fracture reduced the relative survival by 15% (men) and 19% (women), whereas a vertebral fracture reduced the relative survival by 23% (men) 4% (women).

In summary, these data indicate that the post-fracture risk of death in men was greater than in women, and that most of the excess of mortality was associated with hip and vertebral fractures. These results also indicate that the first 12 months after a fracture is an "ideal" time of intervention for reducing mortality.

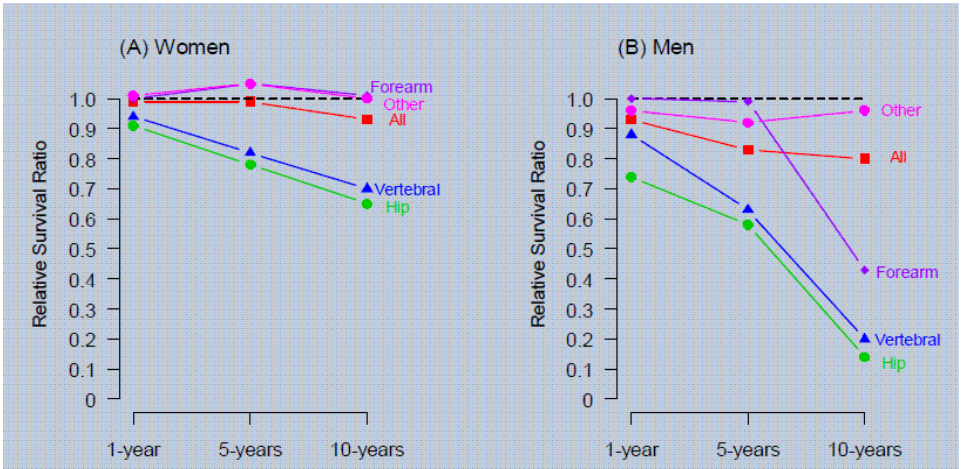

Figure. Relative survival plots by fracture group, women (panel A) and men (panel B).

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MISDIAGNOSIS OF OSTEOPOROSIS BASED ON A SINGLE BONE MINERAL DENSITY MEASUREMENT.

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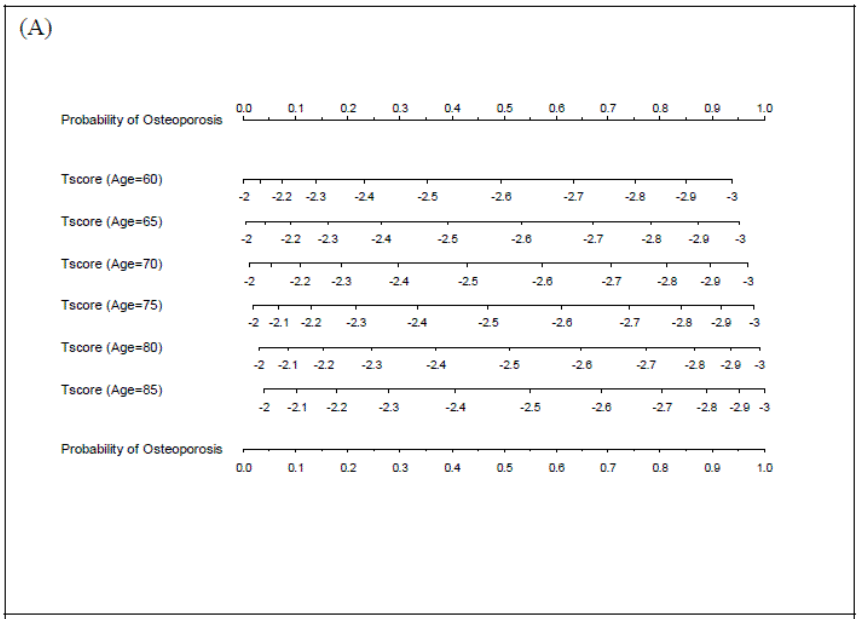
A bone mineral density (BMD) T-score at or below -2.5 is considered to be osteoporotic However, the uncertainty of BMD measurement can result in a false positive or a false negative diagnosis. In this study, a Bayesian approach was developed to estimate the probability of having osteoporosis for an individual woman or man based on the individual's BMD measurement.

An individual's long-term (or "true") BMD measurement is the weighted average of the individual's observed measurement and the population measurement from which the individual is sampled from, with the weight being measurement error and the variance of population BMD. In this study, the measurement error of BMD was determined by a reliability study in which BMD 25 individuals whose BMD was measured twice. The population variance of BMD was obtained from the Dubbo Osteoporosis Study (DOES). Sex-specific nomograms for the prognosis of osteoporosis as a function of age and observed BMD measurement were developed.

For a single BMD T-score, the probability of osteoporosis increased with advancing age (see Figure). A remarkable result of this analysis is that an individual with an observed T-score of -2.5 has only 50% chance of being truly osteoporotic. Even a 60-year old woman with a femoral neck BMD T-score being -2.7, her probability of having osteoporosis is only 61% (ie ~1 in 3 women may be misclassified as having osteoporosis). In women and men with a single femoral neck BMD T-scores of -3.0, the probability of misdiagnosis of osteoporotic is below 5%.

This analysis has shown that due mainly to random measurement error, the diagnosis of osteoporosis based on a single BMD measurement can result in a significant misclassification. The nomogram developed here can help reduce the chance of misdiagnosis.



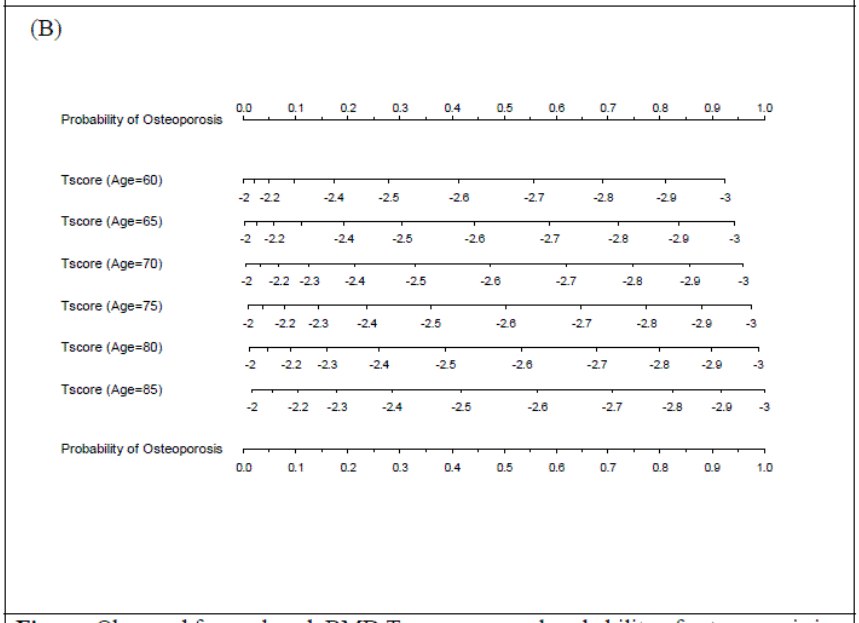


Figure. Observed femoral neck BMD T-score, age and probability of osteoporosis in women (panel A) and men (panel B).

AT 5 YEARS OF AGE DAILY ACCELEROMETRY COUNTS WERE ASSOCIATED WITH LEAN MASS, APPENDICULAR LEAN MASS, AND TOTAL BODY BMC IN BOYS BUT NOT GIRLS: THE FLAME BIRTH COHORT STUDY.

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Weight-bearing physical activity is considered to play an important role in strengthening the growing skeleton. However, few studies have examined relationships between objective measures of physical activity and bone mass in young children. The present study was undertaken to determine whether habitual physical activity assessed by accelerometry in preschool children showed any association with body composition or bone mass measured by dual energy x-ray absorptiometry (Lunar DPX-L). Actical accelerometers were worn sleeping and waking for six days. Satisfactory data were obtained from 158 participants (64 girls, 94 boys) from a New Zealand cohort born between July 2001 and January 2002 who were studied close to their fifth birthday. Girls and boys had similar heights, weights

and average daily accelerometry counts. However, boys had lower fat mass, and higher lean mass and total body bone mass than the girls (P< 0.001). Accelerometry counts showed no associations with height. Daily accelerometry counts were positively associated with lean mass (r = 0.23, P < 0.03), appendicular lean mass (r = 0.25, P < 0.01), total body BMC (r = 0.24, P < 0.02) and total body less head BMC (r = 0.27, P < 0.009) in the boys, but not in the girls (r values 0.03, 0.03, 0.08 and 0.11, respectively). We suggest that greater participation in vigorous physical activity by the boys than the girls may explain these findings. Even at this young age physical activity may need to be vigorous, rather than moderate, to significantly augment muscle gain and bone mass.

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DECAY OF CORTICAL BONE STRUCTURE IN MALES WITH PROSTATE CANCER TREATED WITH 12 MONTHS ANDROGEN DEPRIVATION THERAPY

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Introduction: Sex steroids are important regulators of bone remodelling. In males, the use of androgen deprivation therapy (ADT) for treatment of prostate cancer reduces bone mineral density (BMD), but the structural basis of the deficit in BMD has not been determined prospectively.

Aim: We hypothesized that ADT will reduce cortical and trabecular thickness, increase cortical porosity and reduce serum testosterone levels in males treated for non-metastatic prostate cancer. Here we present preliminary data from an ongoing longitudinal study.

Methods: Preliminary data is presented for 14 males with baseline, 6 and 12 months data after commencing ADT. Assessment comprised medical history, physical examination and fasting blood analyses including sex-hormones. BMD was determined by dual energy x-ray absorptiometry (DEXA) and microarchitecture was assessed using high resolution peripheral quantitative CT (HR-pQCT).

Results: The mean age of subjects was 70 years at baseline. After 12 months of ADT, there was a decrease in total testosterone levels (13.22 to 0.67 nmol/L, p<0.001), but no change in BMI or body weight. BMD decreased at both the lumbar spine (-3.5%, p=0.002) and the total femoral neck (-3.0%, p=0.001). Microarchitecture changed at the distal tibia with a decrease in average bone density (-4.0%, p<0.001), due to a decrease in cortical density (a surrogate of porosity increase) (-2.8%, p<0.001) and cortical thickness (-9.4%, p<0.001). Trabecular density however, remained unchanged. At the radius there was a decrease in average bone density (-5.0%, p=0.006), again due to a decline in cortical density (-2.2%, p=0.004) and cortical thickness (-9.0%, p=0.002) not trabecular density.

Conclusions: Within the constraints of the sample size we infer that bone loss associated with ADT is predominantly due to decay of cortical bone. Absence of trabecular changes may relate to the lower precision of this measurement .

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GLUCOCORTICOID AND TUMOR NECROSIS FACTOR-A ADDITIVELY UP-REGULATE RANKL MRNA EXPRESSION IN HUMAN OSTEOBLAST-LIKE CELLS: INFLUENCE OF GLUCOCORTICOID TREATMENT ON GENERALIZED OSTEOPOROSIS IN RHEUMATOID ARTHRITIS.

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Rheumatoid arthritis (RA) is frequently complicated by peri-articular and generalized osteoporosis due to increased bone resorption. Pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) are thought to be directly responsible for this osteoporosis of RA. In contrast, glucocorticoids commonly prescribed in RA due to their strong anti-inflammatory effect are also well known for causing secondary osteoporosis during a prolonged use. In addition, glucocorticoid and TNF-α have also been reported to induce RANKL mRNA expression in osteoblasts and promote osteoclastogenesis. However, little is known about cooperative effects of glucocorticoid and TNF-α on RANKL expression in osteoblasts. The purpose of this study was to clarify the regulation of RANKL mRNA expression by glucocorticoid and TNF-α in human osteoblast-like cells. We used human osteoblast-like cell line MG-63. Taq-Man real-time quantitative PCR method revealed that 100 nM dexamethasone (DEX) significantly increased RANKL

mRNA expression (15-fold, $p<0.05$) in MG-63 cells for a time-dependent manner. Moreover, 10 ng/ml TNF-α also induced RANKL mRNA expression (3-fold, $p<0.05$) in a dose-dependent fashion. In addition, DEX additively up-regulated TNF-α-induced RANKL mRNA expression. Furthermore, we found that DEX did not influence RANKL transcriptional activity by reporter gene assay using human RANKL promoter. Furthermore, treatment with actinomycin D and DEX markedly prolonged the half-life of RANKL mRNA, as compared to treatment with actinomycin D alone (T1/2 = over 24h v.s. 10h), presumably indicating that DEX-induced RANKL mRNA expression is due to the stabilization of RANKL mRNA. In contrast, TNF-α clearly induced RANKL transactivation and did not influence the stabilization of RANKL mRNA, suggesting that TNF-α regulates RANKL mRNA expression mainly via RANKL gene transcriptional activation. In conclusion, we showed that RANKL mRNA expression was additively up-regulated by TNF-α via RANKL transcriptional activation and by glucocorticoid via the stabilization of RANKL mRNA. Thus, glucocorticoid and TNF-α in inflammatory arthritis such as RA cooperatively deteriorate generalized osteoporosis through two different pharmacological mechanisms of glucocorticoid and TNF-α.

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THE MDCT EVALUATION OF HUMAN FEMORAL BONES SYMMETRY IN AGING, USING QCT AND ANTHROPOLOGICAL MEASUREMENTS: A CROSS-SECTIONAL MORTUARY BASED STUDY.

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Aim: The aim of this study was to measure gender and age-related changes, and the degree of symmetry between contra-lateral femora. In order to complete this, it was necessary to evaluate the accuracy and precision of a clinical helical CT unit, used in a mortuary setting, for such measurements. Method: We evaluated bilateral femora from 289 individuals ranging between 15-95 years from both sexes (n = 154 males and 134 females). We used cross sectional data from high-resolution multiple detector-row computed tomography (MDCT) investigations routinely performed at the Victorian Institute of Forensic Medicine (VIFM) in Melbourne Australia. The study used digital 3D anthropological measurements, adapted from traditional calliper based methods, and quantitative computed tomography (QCT) analysis. Bone mineral density measurements were calibrated by the use of a bone mineral phantom during image acquisition. Individuals were excluded from the study if there were any radiological signs of non-age related bone disease or pathology. Males and females were further divided into age groups representing those ≤ 50 years (n = 73 males and 57 females), and those > 50 years (n = 82 males and 77 females) for trend evaluation. Bilateral anthropological and QCT data including values for maximum femoral length and neck of femur volumetric bone density (vBMD) were then obtained. Results: The radiological differences between right and left morphological and quantitative features of the femora are small taking into consideration the precision of the MDCT methods used. Conclusion: This work represents a critical step in the development of bilateral archetypal models of human femoral bone aging in a contemporary urban population using state-of-the-art 3D radiological imaging techniques.

			Right long femur length (mm)			Left long femur length (mm)		
			n	Mean	Std Deviation	n	Mean	Std Deviation
≤ 50		Male	73	469.7	23.1	73	470.9	22.4
		Female	57	433.7	19.7	57	434.6	19.4
> 50		Male	82	463.0	25.2	82	463.8	24.9
		Female	77	426.0	21.2	77	427.0	21.2

			Right hip vBMD (gm/cm²)			Left hip vBMD (gm/cm²)		
			n	Mean	Std Deviation	n	Mean	Std Deviation
≤ 50		Male	73	296.8	46.7	73	293.3	44.6
		Female	57	301.1	55.7	57	294.2	55.0
> 50		Male	82	254.3	47.8	82	254.1	47.2
		Female	77	231.3	54.6	77	232.5	53.5

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RISK FACTORS FOR CERVICAL AND TROCHANTERIC HIP FRACTURES - A 10-YEAR FOLLOW-UP STUDY

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The biomechanical origins for cervical and trochanteric hip fractures are different [1,2], but there is only limited information on the risk factors for the different types of hip fractures. Our objective was to evaluate the predictive value of calcaneal QUS, radial DXA and clinical risk factors for cervical and trochanteric hip fractures in a 10-year population-based follow-up cohort of elderly women.

The study population consisted of 1222 women living at home (70-73 years at baseline). Lifestyle, anthropometric and physical activity data were collected [3,4]. Radial BMD was measured by DXA (Osteometer DTX 200). Calcaneal broadband ultrasound attenuation (BUA), speed of sound (SOS) and quantitative ultrasound index (QUI) were assessed (Hologic Sahara).

During the 10-year follow-up, 32 cervical and 21 trochanteric hip fractures were observed. The fractured women were taller ($p<0.05$) and thinner ($p<0.001$) than the non-fractured. They had 9% lower BUA and 7% lower QUI ($p<0.05$) than those without fractures. The cervical fracture group had lower BMI and physical activity ($p<0.05$) than the women without fractures. The trochanteric fracture group had lower BMI ($p<0.05$) and higher coffee consumption ($p<0.05$) than those without hip fracture.

Multivariate analyses revealed that high BMI protected from all hip fractures (OR 0.87, 95% CI 0.80-0.95), and low physical functioning was a risk factor for fractures (OR 2.72, 1.32-5.61). The risk factors for cervical fractures were BMI (OR 0.86, 0.77-0.96) and low physical activity (OR 3.26, 1.37-7.74), whereas high coffee consumption (> 5 cups per day) was a risk factor for trochanteric fractures (OR 4.49, 1.49-13.49), after adjustment by age, calcium intake and medication.

To conclude, BUA and QUI correlated with hip fractures, but other risk factors were more predictive in multivariate analyses. BMI was the primary predictor for cervical but not for trochanteric fractures, whereas high coffee consumption predicted trochanteric fractures. Low physical activity was associated with cervical, but not with trochanteric fractures.

Acknowledgements: This study was financially supported by the National Agency for Technology and Innovation (grant nr. 40463/05).

(1) Pulkkinen et al. (2004) Osteoporos Int 15:274-280
(2) Pulkkinen et al. (2006) JBMR 21:895-901
(3) Korpelainen et al. (2003) Osteoporos Int 14:34-43
(4) Korpelainen et al. (2006) Bone 39:385-91

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BONE MINERAL DENSITY, CALCIUM INTAKE AND GENERAL HEALTH OF A COHORT OF POSTMENOPAUSAL FILIPINO WOMEN

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Introduction: Previous bone mineral density (BMD) studies have suggested that Asian women have lower BMD compared with Caucasian women. In the Philippines, use of a Simple Calculation Osteoporosis Risk Estimation (SCORE)[1] aimed to set reference scores for healthy Filipino women suggested that this cohort should not be compared to the Asian data base. The current study aimed to obtain further baseline bone and dietary intake data on a cohort of Filipino women.

Objective: To measure bone mineral density, investigate blood biochemistry and assess diet in a subset of Filipino post-menopausal women.

Methodology: Bone mineral density was obtained using dual energy x-ray absorptiometry (DXA)(GE Lunar DPX-IQ) and anthropomorphic parameters were measured in a subset of 118 Filipino women at least 5 years post-menopause. Blood samples were taken for blood minerals, haematology, liver and kidney function and nutritional status.

Results: Preliminary results indicate that the women had a mean age of 58 ± 4.8 years (range 48 – 65 years old) and a mean BMI of 24.1 ± 4.13 kg/m^2. Very few women reported use of contraceptives or hormone replacement therapy. Mean age of menopause was 48 years (range 45-55) with menarche occurring at age 13 years (range 10-18). DXA examinations of the femoral neck identified osteoporosis in 4.2 % of women (defined as T-score of < -2.5 SD), and osteopenia in 50.0 % (defined as a T-score between -2.5 to -1.0 SD). Considering BMD at the lumbar spine, 21.2% of women were indentified as being osteoporotic and a further 50.8 % as osteopenic. Blood minerals were within normal ranges. Several of the women had high blood cholesterol. Serum folate and vitamin B12 levels were normal. Mean daily calcium intake using 24 hour recall was estimated to be 372mg/day (range 87-883).

Conclusion: The preliminary results of this study shows an early age of menopause, and a high prevalence of osteoporosis and osteopenia in this cohort of women. These findings may be associated with a low calcium intake as well as other dietary and lifestyle factors.

(1) Torralba TP et al. APLAR J Rheumatol 2004; 7: 30-37.

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BONE DENSITY AND CALCIUM INTAKES IN POSTMENOPAUSAL INDONESIAN WOMEN

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There are little data available on bone density and dietary calcium intakes for postmenopausal women in Indonesia. In this study we measured bone mineral density (DXA), followed by anthropomorphic measurements, blood collection and a 24 hour calcium recall. The study group was a subset of 77 Indonesian women at least 5 years post-menopause.

The women had an average age of 58 (range 49 – 74 years old) and an average BMI of 24.6 (range 17.2 – 29.8). Ca intakes based on a 24 hours food recall ranged from 38 to 964.6 mg/day, with a mean intake of 218.7 mg/day. This is very low compared to recommended daily intakes (US AI = 1200mg/day). Ca intake levels will be confirmed with 3day food frequency diaries at a later date. Serum mineral levels were within normal ranges.

DXA examinations of the femoral neck identified 2.6 % of women to be osteoporotic (defined as t-score of < -2.5 SD), and 52% osteopenic (defined as a t-score of -2.5 to -1.0 SD). At the lumbar spine, 18.2% of women were identified as being osteoporotic and a further 48.1% as osteopenic.

The very high prevalence of osteoporosis and osteopenia may be associated with low calcium intakes among other aspects of lifestyle.

Table 1. Anthropometric and bone density data of a subset of 77 postmenopausal Indonesian women.

Characteristics	Values (range)
Age	58 (49 – 74)
BMI (kg/m^2) (n=77)	24.3 (17.2 – 30.4)
24 hour Calcium recall (mg/ day) (n=74)	218.7 (38.0 – 964.6)
BMD (g/cm^2) (n =77)	
Femoral neck BMD	0.771 (0.525 – 1.032)
Femoral neck BMD T-Score	-3.1 – 1.1
L1-L4 BMD	0.929 (0.654 – 1.246)
L1-L4 BMD T-Score	-3.8 – 1.1
Femoral neck	
Osteoporotic (T-Score < -2.5)	2.6%
Osteopenic (T-Score between -2.5 and -1.0)	53.2%

Normal (T-Score > -1)	44.2%
Lumbar spine	
Osteoporotic (T-Score < -2.5)	18.2%
Osteopenic (T-Score between -2.5 and -1.0)	48.1%
Normal (T-Score > -1)	33.7%

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FAILURE TO PERCEIVE INCREASED RISK OF FRACTURE IN WOMEN AGED 55 YEARS AND OLDER. THE GLOBAL LONGITUDINAL STUDY OF OSTEOPOROSIS IN WOMEN

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Aim: To compare self-perceived risk of osteoporotic fracture among women ≥55 years of age with reported risk factors.

Methods: GLOW is an observational study of women ≥55 recruited by 615 primary physician practices in 10 countries. All non-institutionalized patients who visited the practice within the prior 2 years were eligible. Self-administered questionnaires were mailed (2:1 over-sampling of women ≥65). Respondents rated their perceived risk of fracture vs women of the same age using a 5-point scale from "much lower" to "much higher."

Results: Of the women with no risk factors, 89% believed their risk was the same as or lower than that of women of the same age, whereas the majority of women with risk factors failed to appreciate their increased risk of fracture (Table). Among women diagnosed with osteoporosis, 55% believed they were not at increased risk. One quarter of the 17,938 women with a FRACTURE Index ≥5 perceived themselves at higher risk.

Table. Perceived Risk of Fracture Compared with Women of Same Age

Risk factor	N	Perceived risk of fracture	
		Lower or the Same As	Higher
No risk factor	25,301	89%	11%
History of fracture	13,760	64%	36%
Maternal hip fracture	7199	74%	26%
Parental hip fracture	8941	75%	25%
Weight <125 lb (57 kg)	9142	74%	26%
Smoker	5299	80%	20%
Alcohol >20 units/week	287	77%	23%
Current steroid use	1797	61%	39%
Rheumatoid arthritis	6111	71%	29%
FRACTURE Index ≥5	17,938	75%	25%
Diagnosis			
Osteoporosis	12,429	55%	45%
Osteopenia	9974	75%	25%
Normal BMD	36,031	92%	8%

Conclusion: Most women at elevated likelihood of osteoporotic fracture do not perceive themselves to be at increased risk.

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IS VITAMIN D STATUS A CONCERN FOR THE OVERWEIGHT AND OBESE? RESULTS FROM THE ABERDEEN NUTRITION, SUNLIGHT AND VITAMIN D STUDY (ANSAVID)

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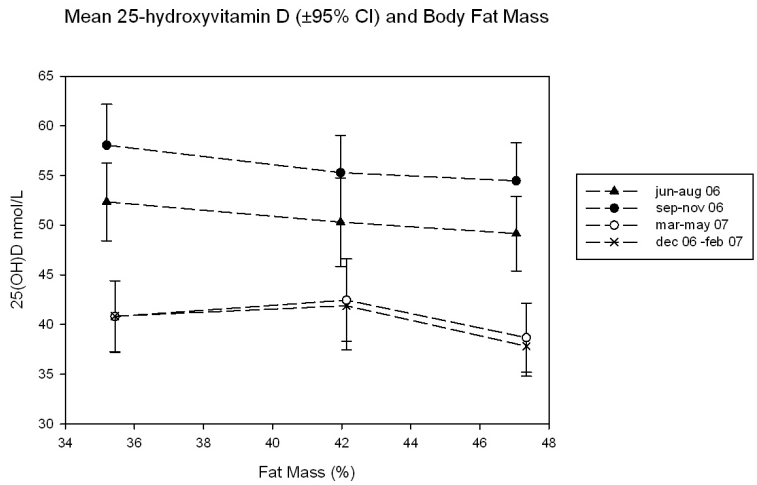
Obesity has been linked with low vitamin D status. We previously observed low serum 25-hydroxyvitamin D [25(OH)D] in women with body mass index (BMI) >30 kg/m^2 (1). Cross-sectional studies in Dutch men and women have shown that 25(OH)D decreases with percentage fat mass (2), explained either by decreased cutaneous synthesis (less sunlight exposure), or reduced accessibility of vitamin D from fat stores. It is not known whether there are seasonal variations or whether the differences in 25(OH)D between individuals with low or high fat mass are clinically significant.

We measured 25(OH)D 3-monthly by enzyme immunoassay (IDS) in Caucasian postmenopausal women (age 55-65y) residing at latitude 57ºN. Total body dual x-ray absorptiometry (Lunar I-DXA) was performed in autumn (October-November 2006, n=330) and spring (March-May 2007, n=310) for assessment of body composition. Fat mass was expressed as % of body weight.

A quarter of women had BMI <25 kg/m^2, 46% between 25-30 kg/m^2, 20% 30-35 kg/m^2 and 9% >35 kg/m^2. Mean %fat mass was higher in spring than in autumn (41.7% and 41.3% respectively, P<0.001). 25(OH)D was inversely associated with weight, BMI and fat mass, significant in autumn (eg fat mass r=-0.11,P<0.04) but not spring (r=-0.09,P=0.10). Mean 25(OH)D decreased with increasing tertile of %fat mass but this was not significant (figure). Women in the top third %fat mass had mean 25(OH)D 3.5 nmol/L (6%) lower than the bottom third in autumn/summer and 2-3 nmol/L (5-7%) lower in spring/winter. Twice as many morbidly obese women had 25(OH)D <25 nmol/L in autumn, winter and spring as women with BMI <35 kg/m^2 (eg spring: 18% vs 9%, P<0.02) but in summer there was no difference (11% vs 9%, P=0.59). There were no differences in 25(OH)D deficiency between obese and non-obese women.



These data indicate that although we see trends similar to other research groups', the differences in 25(OH)D attributable to obesity are small and may not be clinically important.

(1) Macdonald HM, Mavroeidi A, Barr RJ et al Bone 2008;42:996-1003.
(2) van Dam RM, Snijder MB, Dekker JM et al Am J Clin Nutr 2007;85:755-61.

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HIGH BODY MASS INDEX IS ASSOCIATED WITH ENHANCED BONE QUALITY AND REDUCED FRACTURE RISK AT THE DISTAL RADIUS IN MEN AND PREMENOPAUSAL WOMEN

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Evidence suggests that a high body mass index (BMI) is associated with higher DXA-derived aBMD and is protective of fractures. However, the relationship between BMI and bone quality and fracture risk assessed with high-resolution pQCT (HR-pQCT) has not been investigated. Therefore, we aimed to determine whether BMI is predictive of distal radius bone quality and strength and fracture risk. We recruited participants from the Calgary, AB cohort of the Canadian Multicentre Osteoporosis Study (CaMos). This analysis includes 78 premenopausal women, 205 post-menopausal women (93 with no history of hormone replacement therapy (HRT), 112 with a history of HRT use) and 136 men. Participants ranged in age from 20-90 years. We used HR-pQCT (Scanco Medical, Switzerland) and finite element analysis (1) to assess distal radius bone quality and strength (estimated failure load), and we estimated fracture risk using the ratio of the load applied to the wrist during a fall to bone strength [load-to-strength ratio, Φ, (2,3)]. We calculated BMI (kg/m^2) using height and weight and used linear regression to determine the influence of BMI on bone outcomes and Φ after accounting for select covariates including age and bone area. BMI was a positive predictor of volumetric BMD (D100), cortical thickness and trabecular number in men and in pre- and postmenopausal women and of trabecular bone volume ratio (BV/TV) in men only, accounting for 1-6% of the variance in bone outcomes ($p<0.05$). Correspondingly, BMI was negatively associated with trabecular separation in all groups. In postmenopausal women, a high BMI was also predictive of lower trabecular thickness. In men and premenopausal women, BMI was a positive predictor of bone strength (R^2=0.28-0.30, $p<0.01$) and a high BMI was associated with reduced Φ (R^2=0.17-0.25, $p<0.05$). BMI was not predictive of bone strength or Φ in postmenopausal women. Consistent with DXA data, our HR-pQCT results suggest a high BMI is associated with enhanced bone quality. This bone quality advantage confers greater distal radius bone strength and reduced fracture risk in men and premenopausal women. The lack of a protective effect of BMI on HR-pQCT-estimated bone strength and fracture risk in postmenopausal women requires further investigation.

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VARIATIONS IN MECHANICAL LOADING ALTER BONE STRUCTURE DURING GROWTH

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Exercise interventions to improve bone strength have limited effect in adulthood, but positively effect bone mass and strength during growth. To investigate the effect of variations in mechanical loading on bone structure during growth we compared bone structure at the distal tibia on the affected to the non-affected side in 13 children (cases) with altered weight bearing ability due to Legg-Calvé-Perthes disease at the femur (69% males, 54% pre-pubertal, mean age 11.3 ± 0.9 years). These differences were compared to the side-to-side differences in bone structure in 35 sex-, age-, and maturity-matched healthy controls (77% male, 80% pre-pubertal, mean age 10.6 ± 0.4 years). We hypothesized that bone structural parameters would be greater in the non-affected than affected tibia of cases and the side-to-side differences in these parameters would be greater in cases than controls. Bone structure was measured using high-resolution micro pQCT and bone strength (CSMI and section modulus) estimated from these measurements. Anthropometry (height, weight, tibia lengths), pubertal status (Tanner staging), hours of weight-bearing exercise and mean calcium intakes were recorded. Side-to-side differences were determined using paired t-tests, and between group comparisons (cases vs. controls) determined using unpaired t-tests.

Significant side-to-side differences in cortical area (CoA; 8.8 ± 3.2%, $p < 0.01$) and cortical thickness (CTh; 8.5 ± 4.1 %, $p < 0.05$) were observed in cases, but not controls (CoA; -0.3 ± 1.6, CTh; -1.3 ± 0.8, NS). The side-to-side differences in CoA and CTh were 9.1 ± 3.3% ($p < 0.01$) and 9.8 ± 3.8% ($p < 0.05$) greater than the differences in

controls. Cases and controls did not differ by age, height, weight, hours of weight-bearing exercise or daily calcium intakes.

Using this model that controlled for genetic and environmental factors that may influence bone growth, these observations support the notion that mechanical loading during growth alters bone structure. If changes are permanent then exercise during growth may be a feasible option to reduce bone fragility and fracture risk in later life.

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COMPLEMENTARY AND ALTERNATIVE MEDICINE USE BY PATIENTS WITH OSTEOPOROSIS IN AUSTRALIA (CAMEO-A) STUDY

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Complementary and alternative medicine (CAM) therapies have become increasingly popular and are used regularly by patients with osteoporosis. The prevalence and characteristics of CAM use by patients with osteoporosis in Australia is unknown.

We performed a prospective, questionnaire-based study to determine the prevalence and patterns of use of CAM therapies in 202 outpatients with osteoporosis (mean age: 68.5 ± 10.9 years; 79.7% of female gender; mean serum 25-hydroxy-vitamin D level = 66.7 ± 20.3 nmol/L). One-hundred-and-forteen respondents (55.9%) were born overseas out of which 34 (29.8%) were from an Asian country.

CAM use was reported by 104 patients (51.2%). The most frequent were vitamins (24.0%), acupuncture (19.2%), Tai chi (14.4%) and yoga (12.5%). More than one form of CAM therapy were tried by 30.8% of respondents, and 84 (80.8%) thought their osteoporosis improved with these approaches.

Those who used CAM were more often university-educated (25.5% vs 14.4%, $p < 0.05$), with a lower lumbar spine t-score (-2.35 vs -2.20 SD, $p < 0.05$), and were more likely to visit the clinic over a 12 month period (1.8 vs 1.5 times, $p < 0.05$) compared to CAM non-users, with no significant differences in terms of their age, gender, marital status, mean serum vitamin D level, femoral neck t-score or years since diagnosis of osteoporosis.

The most common reasons for using CAM for patients were having a holistic approach to their healthcare (53.0%) and having inadequate pain control (29.0%). More than half (57.4%) of the respondents were unable to differentiate between the terms ‘osteoporosis' and ‘osteoarthritis', especially those from a non-English-speaking background (64.1% vs 46.4%, $p = 0.02$) and those who did not use CAM (57.1% vs 41.0%, $p = 0.01$). It was not associated with patients with inadequate pain control as an explanation for CAM use.

Twenty-three percent of CAM users stated their treating doctors were unaware of their CAM therapies, and 73.3% did not consult a physician before starting CAM.

We conclude that there is a high prevalence of CAM use in our patients with osteoporosis, and the majority of them reported that their symptoms improved. CAM users were better educated and visited their physicians more often than non-users, but most did not discuss CAM treatments with their physician. Physicians reviewing patients with osteoporosis should enquire about CAM use, spend more time on patient education (with the use of an interpreter for non-English speaking patients) about disease mechanism of osteoporosis.

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IS SEASONAL VARIATION IN VITAMIN D STATUS AFFECTED BY HOLIDAYS AND COD LIVER OIL? RESULTS FROM THE ABERDEEN NUTRITION, SUNLIGHT AND VITAMIN D STUDY (ANSAVID)

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Vitamin D may influence many health outcomes. Circulating 25-hydroxyvitamin D [25(OH)D] ≥25 nmol/L is sufficient to prevent rickets but levels of 25(OH)D >40 nmol/L (or greater) have been proposed for optimal health. Healthy adults in the UK obtain most of their vitamin D by exposure of skin to sunlight with seasonality, latitude and lifestyle playing an important role. The aim of this longitudinal study was to assess the seasonal variation of 25(OH)D at 57 º N.

365 Caucasian women [mean age 61.4 y ± 1.5 (SD)], >5y postmenopausal, attended 3 monthly visits over 15 months (with an additional visit at 2y) for fasted serum 25(OH)D (IDS enzyme immunoassay). Women were asked whether they took dietary supplements (cod liver oil) and if they had holidayed abroad in the preceding 3 months.

	Vitamin D status (all women)			Vitamin D status (holiday goers)		
		25(OH)D (nmol/L)	% in cut-off groups <25nmol/L / <40nmol/L		25(OH)D (nmol/L)	% in cut-off groups <25nmol/L / <40nmol/L
	N	Mean (SD)	%	N	Mean (SD)	%
Spring ‘06 (Mar-May)	365	39.4(18.8)	20.9 /60.1	74	53.5(24.5)	5.4/31.1
Summer ‘06 (June-Aug)	338	55.7(20.6)	2.7/23.7	104	63.8(22.5)	0.0/11.5
Fall ‘06 (Sep-Nov)	332	50.6 (21.6)	7.2/33.4	96	56.6(25.6)	5.2/25.0
Winter ‘06/07 (Dec-Feb)	314	40.0(19.3)	22/56.1	52	53.7(23.0)	3.8/32.7
Spring ‘07 (Mar-May)	312	40.5(19.5)	20.5/56.7	58	54.9(25.5)	6.9/27.6
Spring ‘08 (Mar-May)	258	39.7 (19.4)	24.8/60.5	40	49.2(23.3)	19.4/54.7

Mean 25(OH)D was highest in the summer and lowest in spring and winter, with 21-25% of the population below 25nmol/L ($p < 0.001$). Holidays abroad or cod liver oil usage reduced the number of women with vitamin D deficiency ($P<0.01$ for most visits).

These longitudinal data suggest that 25(OH)D varies with season at northerly latitude and that holidays abroad or dietary supplements such as cod liver oil may be important for maintaining vitamin D sufficiency.

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RELATIONSHIP OF QUANTITATIVE ULTRASOUND CALCANEUS MEASUREMENTS TO ACTIVITIES OF DAILY LIVING AND QUALITY OF LIFE IN THE AGED

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Back ground: It is reported that speed of sound (SOS) of calcaneus is correlated with the bone mineral density of lumber vertebra or femoral neck, though it doesn't use as a diagnosis standard of osteoporosis. Quantitative ultrasound (QUS) has the advantage of being easy, fast and cheap in the measurement of bone mass. Because of this, QUS is useful for screening of bone loss in the aged people. This study, therefore, was investigated that relationship of bone mass to activities of daily living (ADL) and quality of life (QOL) in the aged people with QUS.

Subjects and Methods: Subjects were 160 aged people (61-100 years old) in a nursing home. Barthal Index (BI) and Japanese Osteoporosis Quality of Life Questionnaire (JOQOL) scores were used as ADL and QOL assessment,

respectively. SOS of calcaneus was measured with QUS (CM-100). In statistical analysis, Spearman rank correlation coefficient was used to find the relationship of SOS to BI and JOQOL score in all subjects and age groups. Differences in SOS, BI and JOQOL score in age groups and in SOS of locomotion methods were examined with Kruskal-Wallis test or one-way ANOVA followed by Tukey-Kraymer tests. A significance level of p=0.05 was set. This study was carried out in getting informed consent from all subjects.

Results: SOS correlated with BI (r=0.277, p<0.0005) and JOQOL (r=0.439, p<0.005) in all subjects significantly. The interrelation between SOS and BI were shown in70's, 80's and 90's groups. SOS of independent gait subjects was significantly higher than that of subjects with T-cane, walker and wheel chair.

Conclusions: SOS of aged people mutually related with BI score. This was considered because a decline in BI score was caused by locomotion methods and loading to the heels of independent gait was more than that of T-cane gait, walker gait and wheel chair.

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EFFECT OF OFFICE-BASED IMPACT EXERCISE ON BONE IN PREMENOPAUSAL JAPANESE WOMEN: SENDAI BONE HEALTH CONCEPT STUDY

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Aim: Impact exercise has been shown to be beneficial for the bone. However, the effect of impact exercise on bone has not been shown in Japanese premenopausal women. Methods: We performed a 12-month randomized, controlled office-based brief exercise trial in 91 Japanese premenopausal women. Participants were randomly allocated into the following 2 groups: low-impact exercise group (LIE, 46 women, exercise program: Tai-Chi and stretching exercise, 10~15 min/session, 3-5 sessions/week) and high-impact exercise group (HIE, 45 women, exercise program: 5 x 10 (max) jumps progressively inserted during LIE; 10~15 min/session; 3-5 sessions/week). Lumbar spine and proximal femur BMD were measured using DXA (Hologic QDR-4500). ANCOVA was used for the analyses, adjusting for change in BMI and dietary Ca intake. Results: Thirty-three (72%) women in the LIE and 34 (76%) in the HIE group completed the study. The mean rate of attendance to the exercise program was 2.39 times/week for all participants. No accidents or injury related to the exercise program were reported. The mean (• }SD) baseline calcium intake was 462.4• }150 mg/d in the LIE and 445.5• }172.1 mg/d in the HIE group. There was a similar, statistically significant increment in energy-adjusted Ca intake over the trial in both groups. Leg strength also increased significantly in both groups (28.0% in LIE and 29.5% in HIE). Femoral neck bone mineral density (BMD) decreased 1.0% in the LIE group while it increased in 0.6% in the HIE (p<0.05 between the groups). Furthermore, L1-L4 BMD increased in the HIE (p<0.05) and decreased in the LIE but the difference between the groups was not significant (p=0.14). Conclusion: Although Ca intake did not reach Japanese standard dietary recommendation (800mg/d), regular office-based brief exercise including jumping appears to have positive effect on BMD in premenopausal Japanese women. The effect seems to be most pronounced at the femoral neck. Even low impact exercise increases leg strength which might be beneficial in maintaining their physical activity. In conclusion, office-based exercise can be recommended for Japanese women for prevention of osteoporosis.

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TRABECULAR ROD AND PLATE MORPHOLOGY IMPROVES PREDICTION OF VERTEBRAL BODY BONE STRENGTH BEYOND APPARENT BONE DENSITY

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Micro-CT imaging enables accurate three-dimensional representation of bone microarchitecture and subsequent mechanical testing of the same specimen allows measurement of bone strength. Based on micro-CT data it is now also possible to perform morphometric analysis on individual rod and plate bone trabeculae using a volumetric spatial decomposition algorithm and hence determine the contribution of these individual elements to bone strength.

Twelve pairs of vertebral bodies (T12/L1 or L4/L5) were harvested from 12 human cadavers and bone cubes (10mmx10mmx10mm) were obtained. After micro-CT imaging, the volumetric spatial decomposition algorithm was applied. Trabeculae were classified as rods or plates and trabecular rods were further classified as horizontal, vertical or oblique. Mean rod and plate thickness (< Ro.Th > and < Pl.Th >), mean rod and plate length (< Ro.L > and < Pl.L >), mean horizontal, vertical and oblique rod thickness (< Ro_H.Th >, < Ro_V.Th >, < Ro_o.Th >), mean horizontal, vertical and oblique rod length (< Ro_H.L >, < Ro_V.L >, < Ro_o.L >) and BV/TV were calculated for each specimen. Bone strength was measured in compression, were one specimen from each vertebral body pair was tested supero-inferiorly (on-axis) and the paired specimen was tested antero-posteriorly (off-axis).

BV/TV was the strongest single predictor of on-axis (r^2=0.77, p<0.0001) and off-axis strength (r^2=0.54, p<0.0001), respectively. In multi-regression analysis, prediction of on-axis strength was improved to r^2=0.90 with the addition of < Ro.L >, < Pl.Th > and Pl.BV/TV and to r^2=0.87 with the addition of < Ro_V.Th >, < Ro_V.L > and < Pl.L >. Prediction of off-axis strength was improved to r^2=0.92 with the addition of < Ro.V >, < Ro.L > and the ratio of < Ro.V > to < Pl.V > and to r^2=0.83 with the addition of < Ro_H.L >, < Pl.Th > and < Pl.L >.

Microarchitectural measures of individual trabeculae that contribute to bone strength have been identified. In addition to the contribution of apparent bone density, measures of trabecular rods contribute up to an additional 38% to prediction of off-axis strength, whereas measures of trabecular rods and trabecular plates contribute up to an additional 13% to prediction of on-axis strength. Decomposing vertebral body bone architecture into its constituent elements enables identification of the critical components that determine bone strength, which might offer new avenues in fracture risk prediction in patients.

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AGE-DEPENDENT FEATURES OF BONE TISSUE STATE IN UKRAINIAN MEN

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This research was aimed to studying the age-dependent peculiarities structural-functional state of bone mass in Ukrainian men.

Object. 1200 men are inspected in age from 20 to 88 years ((M ± m): age - 54,1±0,5 years; height - 1,74± 0,003 m ; weight - 81,6± 0,4 kg) were examined and divided into the following age-dependent groups: 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80-89 years old.

Methods . The structural-functional state of bone mass was determined using ultrasound densitometry with use of "Achilles+".

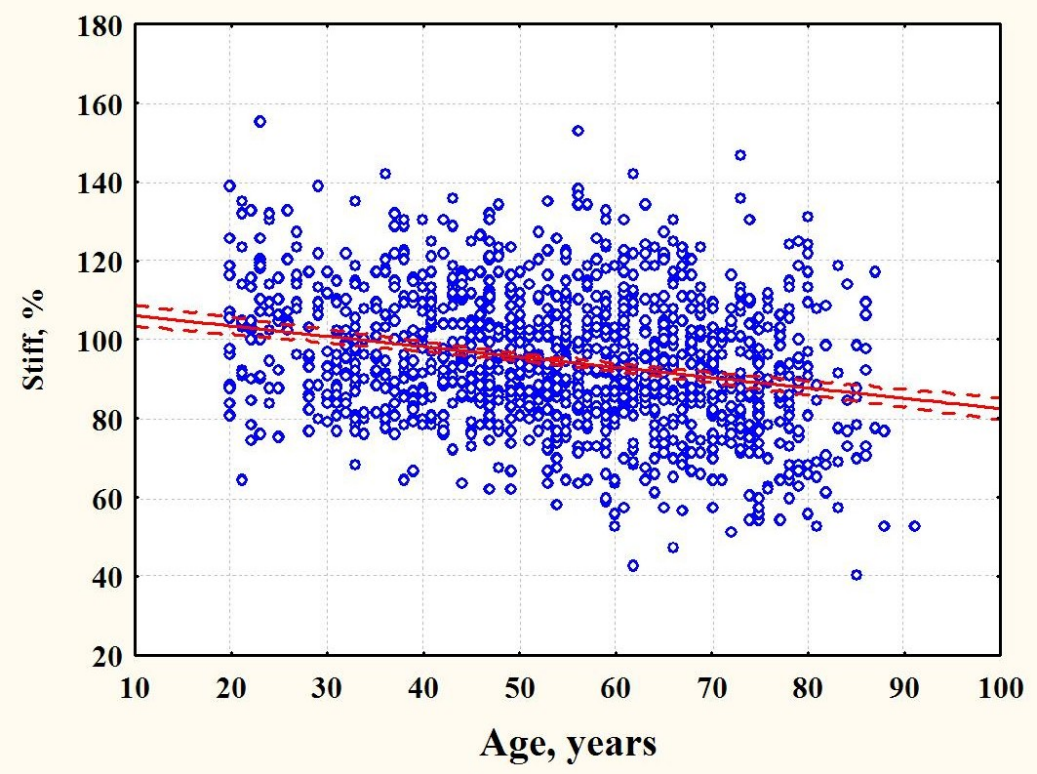
Results. M ineral density of bone in dependence on age are presented in table.

Parameters	20-29 years	30-39 years	40-49 years	50-59 years	60-69 years	70-79 years	80-89 years
n	10 2	1 55	2 14	2 36	2 64	1 79	50
Age, years	24.2±0.3	34.7±0.2	44.9±0.2	54.9±0.2	64.3±0.2	74.3±0.2	83.1±0.4
Height , m	1.78±0.008	1.78±0.006	1.7 6 ±0.005	1.74±0.004	1.72±0.004	1.70±0.005	1.70±0.009
Weight , kg	76.0±1.3	82. 3 ±1.1	85.5±1.0	84.5± 1 .0	82.1±0.9	77. 2 ±0.9	73.3±1.4
BMI, kg/m^2	23.8±0. 4	26.2±0.3	27.7±0. 3	27.6±0. 3	27.7±0. 3	26.7±0. 3	25.4±0.5
SOS , m/s	1579. 2 ±4. 1	1561.6±2. 3	1561. 6 ±2. 3	1554.0±2. 3	1547.6±2. 2	153 7.6 ±2.7	1531.1±6.3
BUA , dB/MHz	122.7±1.1	118. 3 ±0.9	12 0 . 7 ±0.8	119.7±0.7	119.3±0.7	11 5 . 8 ±0.9	115.2±2.1
S I, %	104.0±1. 7	96. 4 ±1.2	98. 0 ±1. 1	95.1±1. 1	92. 8 ±1. 3	8 7 . 7 ±1.3	85.7±3.1

T -score, SD	0.3 7 ±0.15	-0.3 6 ±0.11	-0.2 2 ±0.09	-0. 47 ±0.09	-0. 66 ±0.09	-1.1 2 ±0.11	-1.3 4 ±0.28
Z -score , SD	0.57±0.15	0.38±0.11	0.96±0.10	0.78±0.09	0.61±0.09	0.13±0.12	-0.07±0.27

Notes: results are represented as M ± m, BMI - body mass index, SOS – speed of sound, BUA – broadbound ultrasound attenuation, SI – stiffness index .

With age the gradual diminishing of indexes of the structurally-functional state of bone fabric is set at men. The reliable decline of indexes of SOS and SI is observed at men after 50 years, and reliable decline of BUA - at men more senior 70 years. Age had a rather weak negative impact on SI described by a linear model (SI=108.7-0.26*Age; r= -0.25. t=8.9. p<0.00001) (Fig.1).



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BONE MINERAL DENSITY IN UKRAINIAN WOMEN

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Objective . The aim of this study were: to determine spine, femoral and radial BMD for a representative sample of healthy women of Ukrainian female descent, to determine the effect of age, height and weight on BMD, and to compare these results with those from a large USA/Northern Europe and US/European reference sample.

Materials and methods. The research was conducted at the Ukrainian Scientific-Medical Centr e for the Problems of Osteoporosis , and included 353 women aged 20-79 years. Conventional BMD measurements of the spine (L1-L4 in the anterior-posterior position), proximal femur (neck, Ward's triangle and trochanter regions) and radial shaft (33% site) were determined by DXA using a densitometer Prodigy (GE Medical systems).

Results. Age-related changes in BMD were similar in form to those of USA / Northern Europe and US/European reference data. However, BMD of spine f or subjects of 50-59 years in our sample were lower than published values . Regression analyses showed that weight was a significant predictor of female spine and femur BMD for both the premenopausal and postmenopausal decades. Age was a significant predictor of female spine BMD in the 50-79 year age. The prevalence of osteoporosis and osteopenia for female subjects was 11% at the femur neck, and 20% and 24% at the spine and radial shaft respectively. Substantially lower prevalence of osteoporosis of lumbar spine in Ukrainian population, based on the WHO criteria, was established in comparison with US/European reference values.

Conclusion .Thus , standardizing of BMD measurements by DXA through the appropriate use of population-specific reference values is recommended to improve the quality of medical care provided in relation to the prevention and treatment of female subjects who are at risk as for osteoporosis or are already osteoporotic.

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BONE MINERAL DENSITY ACCORDING TO ANSWER IOF'S ONE-MINUTE OSTEOPOROSIS RISK TEST

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This research aimed at evaluating the bone mineral density according to answer IOF's one-minute osteoporosis risk test.

The study included two stages. Test was translated into Ukrainian. At the first stage, structural-functional state of bone was evaluated by means of an ultrasound bone densitometer ("Achilles+") . We've examined 147 postmenopausal women aged 50-69 years (mean age 59,8 ±0,7). The speed of sound (SOS, m/s), broadband ultrasound attenuation (BUA, dB/MHz) and "Stiffness" index (SI, %) were measured. Parameters of u ltrasound densitometry at patients who have answered positively on II (Have you broken a bone after a minor bump or fall), III (Have you ever taken corticosteroid tablets for more than 3 consecutive months) and IV (Have you lost more than 3 cm in height) questions, were significantly less in comparison with the patients who have answered negatively. SI at patients with the positive answer to the on II the question has made 74,0±1,7 %, with negative - 81,2±1,3 %, p = 0,002; on III - 67,1±3,9 % and 79,9±1,1 %, p = 0,0013; on IV - 71,6±1,7 % and 82±1,2 %, p <0,00001. Rate of osteoporosis depending on the positive answer to the following questions has been made: to the on II question - 46,67 %, to the on III - 81,82 %, to the on IV - 58,1 %. At the second stage of BMD, T and Z-score of the spine, femoral neck were determined by DXA using a densitometer Prodigy (GE Medical systems). We've examined 73 postmenopausal women aged 50-69 years (mean age 63,9±0,9). Significant correlation between the answer to the on II a question and BMD spine (r =-0,29; p=0,012) and BMD femoral neck (r =-0,32; p=0,005); between the answer to the on IV a question and BMD spine (r=0,29; p=0,047) was found.

Application of IOF's one-minute osteoporosis risk test gives an opportunity to determine structural-functional changes of bone.

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EFFECT OF PEPTIDE REGULATORS ON STRUCTURAL AND FUNCTIONAL STATUS OF OSSEOUS TISSUE IN AGEING

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Our study was aimed at evaluating the effect of peptide bioregulators on the structural and functional status of osseous tissue in a post-ovariectomy osteoporosis model in rats. 100 mature female Wistar rats aged 4-6 months with body weight of 200- 230 g were randomly subdivided into 8 groups, each consisting of 10 rats, and received the studied substances intramuscularly in different doses, the control being made up of 2 groups of 10 rats — ovariectomized animals not treated with substances, and non-operated animals injected with physiological NaCl solution. The following peptide bioregulators were used in the study: substance extracted from cartilages of young calves, in the dose of 1 mg and 0,03 mg per rat, and peptide medication T-31 (H-Ala- Glu-Asp-OH) in the dose of 10 µg and 0,3 µg per rat. To model the post-menopausal osteoporosis, bilateral ovari ectomy was performed. Mineral density of the osseous tissue (MDOT) was evaluated using a two-photon X-ray densitometer «PRODIGY». Study results pointed out the reliable efficacy of cartilages extract and T-31 peptide in maximum dosages in case of their administration from the 30^{Ih} day since ovariectomy operation. The strongest effect was observed in case of cartilages extract administration in the maximum dosage (1 mg per rat): after a month of observation MDOT was reliably increased, remaining on the same level after 2 months since the beginning of the experiment. The administration of T- 31 in the maximum dose beginning immediately after ovariectomy caused a reliable increase in MDOT after 30 days. Thus, peptide bioregulators show good prospects as a means of prevention and treatment of post-menopausal osteoporosis.

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RELATIONSHIP OF HORMONAL STATUS AND BONE STATE IN MEN

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The aim of the study was to determine the relationship of hormonal status and bone state in men.

We have examined 96 men aged from 30 to 79 years (M±m): age - 54,4±1,3 years; height - 1,75 ± 0,01 m ; weight - 84,9 ± 1,5 kg), divided them into age dependent subgroups 30-49 (n =36; age - 41 , 2 ±1, 2 years) and 50-79 years (n =60; age - 64 , 4 ±1, 1 years) . Levels of testosteron (Test, nmol\l) and sex hormone - binding globulin (SHBG, nmol\l) were determined by means of chemiluminescent immunoanalysis method. The bone mineral density (BMD, g/cm^2) was evaluated for the total body, spine (L_1-L_4), femur (neck, t rochanter and total) and radius (ultradistal, 33% and total) using dual energy x-ray absorptiometry by the P rodigy instrument (GE Medical systems, 2005).

The correlation analysis of age dependent sub-groups: in the group of 30-49 years there is a positive correlation between Test and BMD ultradistal radius (r=0,49, p < 0,05), along with the negative correlation between SHBG and Total body in the group of 50-79 years (r=-0,31, p < 0,05). In the group of 60-79 years (n = 38 ; age - 6 9,7±1,0 years) we have found a negative correlation between SHBG and Total body (r=- 0, 60 , p < 0,0 01) , SHBG and t rochanter (r=- 0,47, p < 0,05), SHBG and Total femur (r=- 0,48, p < 0,05).

Patients of 50-79 year age group with normal bone, osteopenia and osteoporosis were chosen in correspondence to the WHO criteria . For analysis' sake, we have joint the osteopenia and osteoporosis patients. Normal mineral density of lumbar spine was found in 83,3%, osteopenia and osteoporosis – 17 ,7%, while in total femur – 75% и 25% respectively . SHBG in normal femur BMD subgroup (41,1 ± 2, 6) was considerably lower than in osteopenia and osteoporosis subgroup (54,4 ± 5 , 6, p < 0,05).

Thus , we have revealed a positive correlation between testosterone levels and ultradistal radius BMD and negative correlation between SHBG and total body BMD in patients of 50 – 79 year age group, t rochanter and total femur in patients of 60 – 79 year age group.

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ULTRASOUND DENSITOMETRY EVALUATION IN POSTMENOPAUSAL WOMEN WITH COLLES' FRACTURE

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This research was aimed at studying the bone tissue state among women with Colles' fracture by means of the ultrasound densitometry method. The total of 34 healthy postmenopausal women 42 – 74 years old (62,1 ± 7,5) with Colles' fracture in their anamnesis (CF) were examined by ultrasound bone densitometer “Achilles+” (Lunar Corp., Madison, WI). The control group included postmenopausal women without any osteoporotic fractures in their anamnesis (WF), being standardized by age, BMI, etc. The speed of sound (SOS, m/s), broadband ultrasound attenuation (BUA, dB/MHz) and a calculated “Stiffness” index (SI, %) were measured. The main risk factors for the osteoporotic Colles' fracture turned out to be a menarche after 15 years, an early and late menopause. 29,3% of patients with Colles' fractures had a bone tissue Stiffness index coinciding with the baseline of fracture risk or under it.

There was no revealed relation among the age and the ultrasound densitometry indices among women of posmenopausal age without fractures. Only 12,5% of patients with Colles' fractures were noticed to have a normal bone tissue.

The ultrasound parameters were veritably lower among postmenopausal women with CF than among WF (SOS: CF – 1524 ± 28,4; WF – 1543 ± 24,3, p < 0,05; BUA: CF – 102 ± 17,8; WF – 109 ± 12,0, p < 0,05; SI: CF – 76 ± 14,9; WF – 85 ± 13,5, p < 0,05; all values are the mean ± SD). It is caused by the decrease of bone tissue mineral density, accelerated aging, and the development of osteopaenia and osteoporosis.

The most tangible differences in these indices were noticed among the elderly patients. Colles' fracture indicates osteopaenia and osteoporosis in postmenopausal period. In summary, ultrasound densitometry is an effective screening method to reveal the women of risk group with future osteoporotic Colles' fracture in postmenopausal period.

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THE RISK OF UPPER GASTROINTESTINAL (GI) ADVERSE EVENTS OF PATIENTS SWITCHING FROM RISEDRONATE TO ALENDRONATE AFTER THE INTRODUCTION OF GENERIC ALENDRONATE PRODUCTS IN THE UK

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Since their introduction in 2005, generic alendronate products have become the most widely prescribed osteoporosis therapy in the UK. In an effort to reduce costs, many health care providers initiated "switch programs" to generic alendronate for patients on other oral bisphosphonates. Recent exploratory retrospective studies suggest differences in upper gastrointestinal tolerability between generic alendronate, branded alendronate, and risedronate. This retrospective cohort study evaluated if patients stabilized on risedronate who switched to alendronate have an increased risk for upper GI events.

Using the UK THIN (The Health Improvement Network) database, between March 2005 and July 2007, 3,492 patients (50+ years) with a new prescription for weekly risedronate (RIS) and/or alendronate (ALN), with a 2 year washout period, were selected for analysis. Study cohorts were defined as 1) stabilized RIS users (duration ≥ 6 months, no upper GI events during the first 6 months), 2) stabilized RIS users who switched to ALN therapy (≤ 45 days from the date of last RIS prescription), maximizing the likelihood of switching due to cost reduction. Calendar year was used as a proxy of generic alendronate availability. A multivariate Cox regression model was used to evaluate the risk of upper GI events within the first 6 months of treatment.

Of the RIS patients 80.2% were female and 44.9% switched to alendronate in 2007, compared to 1.7% in 2005. Overall, stabilized RIS users who switched to ALN had an 85% significantly higher risk of any upper GI events compared to RIS only patients. A 76% significantly higher risk was found in those without any history of upper GI events.

Findings from this retrospective cohort study suggest an increased risk of upper GI events in patients stabilized on risedronate who switch to alendronate after the introduction of generic alendronate products in the UK.

Study cohorts	Incidence rate (person-year)	Hazard ratio* (95%CI) Full cohort (n=3492)	Hazard ratio* (95%CI) No history of upper GI (n= 3128)
RIS - RIS (n=2962)	0.121	1.00	1.00
RIS - ALN (n=530)#	0.165	1.85 (1.26 – 2.72)	1.76 (1.15 – 2.69)
*Multivariate Cox regression model adjusted for history of upper GI events and concomitant PPI/H2 blocker use #Duration on RIS therapy before switch (mean ± standard error): 1.77 ± 0.03 years			

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MOTHERS WITH PREGNANCIES COMPLICATED BY UTEROPLACENTAL INSUFFICIENCY DO NOT HAVE IMPAIRED BONE MINERAL CONTENT, DENSITY OR STRENGTH DURING AND AFTER LACTATION

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Background and Aims: Uteroplacental insufficiency is a complication arising in 10% of human pregnancies and is characterised by reduced placental blood flow, with a reduction of oxygen and nutrient supply to the fetus resulting in intrauterine growth restriction. We have previously shown in the rat that pups born to mothers with uteroplacental insufficiency are born of low birth weight, have lower total body calcium content and consume milk with lower calcium as a consequence of reduced placental and mammary calcium transfer, respectively. As adults, offspring born small have bones which are smaller and weaker than controls. It is established that there are losses of calcium from the maternal skeleton during lactation to support skeletal development in the offspring. The aim of this study was to determine whether the bones of mothers with uteroplacental insufficiency would be more adversely affected during lactation when compared to controls.

Study Design: To induce uteroplacental insufficiency, bilateral uterine vessel ligation (Restricted) or sham surgery (Control) was performed on gestational day 18 (term=22 days) in WKY rats. Post mortem of Control and Restricted mothers was performed on day 1, day 35 (weaning), 7 weeks and 9 weeks post partum. The right femur was dissected and f emur length, dimensions, mineral content and density were measured at both trabecular and cortical sites using peripheral quantitative computed tomography (pQCT).

Results: Restricted pups were born lighter than Controls ($p < 0.05$). There were no differences in maternal body weight between the Control and Restricted groups at any time point. Femur trabecular and cortical mineral content and density were not different between groups. Measures of bone geometry, including periosteal and endosteal circumferences, as well as cortical thickness were not different between groups. The stress strain index of bone bending strength was also not different when comparing the Control and Restricted groups at all time points studied.

Conclusions: These results suggest that mothers with pregnancies complicated by uteroplacental insufficiency do not have a bone deficit. Maternal bone homeostasis is unable to compensate for the adverse consequences of uteroplacental insufficiency on placental and mammary calcium transfer to the growth restricted offspring.

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MULTINATIONAL COMPARISON OF BONE HEALTH IN WOMEN 55 YEARS OF AGE AND OLDER. THE GLOBAL LONGITUDINAL STUDY OF OSTEOPOROSIS IN WOMEN

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Aim: To compare bone health among women ≥55 years from three geographic regions.

Methods: Practices typical of each region were identified through primary care networks organized for administrative, research or educational purposes. All non-institutionalized patients visiting each practice within the prior 2 years were eligible. Self-administered questionnaires were mailed, with 2:1 over-sampling of women ≥65 years of age. Follow-up questionnaires will be sent at 12-month intervals for 5 years.

Results: The study population comprises 60,393 women from 615 physician practices in Europe, USA and Canada/Australia. Twenty-three percent of women reported at least one fracture occurring after age 45: 17% had a single fracture and 6.0% had two or more. The most frequently reported fracture was of the wrist (8.6%) followed by the ankle (6.2%; Table). Overall, 11% of fractures affected weight-bearing bones of the lower body, which are associated with greater levels of disability. Twelve percent of women said that their mothers had sustained a hip fracture. Across study sites, age-standardized prevalence of previous fractures ranged from 22% in the USA to 25% in Europe. Wrist fractures were reported by 10% of women in Europe but only 7.1% of US women; hip fracture reports were highest in Europe (2.0%) and lowest in Canada/Australia (1.4%).

Table. Women reporting fracture by geographic region (age standardized)

Fracture site (%)	Europe N=25,334	USA N=28,170	Canada/Australia N=6889
Clavicle	1.5	1.2	1.4
Arm	3.3	2.9	2.6
Wrist	10	7.1	8.6
Spine	2.8	1.9	2.0
Rib	4.6	3.9	4.2
Hip	2.0	1.9	1.4
Pelvis	1.0	1.2	0.8
Ankle	5.7	6.8	6.1
Upper leg	1.0	1.1	0.6
Lower leg	2.5	2.6	2.3
Upper body	18	14	16
Lower body	11	12	9.9

Conclusions: Fracture incidence data gathered in subsequent years of the GLOW study will provide a better assessment of whether there is significant variation in the incidence of certain fracture types

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MENSTRUAL IRREGULARITY IS ASSOCIATED WITH HIGHER BONE MASS IN YOUNG WOMEN WHICH IS MEDIATED THROUGH ALTERATIONS IN ENDOGENOUS ANDROGENS

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Background: There have been few studies examining the association between menstrual irregularity, androgens and bone mineral density in premenopausal women.

Objective: To describe the association between menstrual irregularity, androgens and bone mass measured by quantitative ultrasound (QUS) in premenopausal women who were not taking hormonal contraceptives.

Method: Australia wide cross-sectional study (N=382, mean age 31 years). Menstrual irregularity was assessed based on self-administered questionnaire. Bone mass was measured by Hologic Sahara densitometer. Total testosterone and SHBG were assessed by fasting blood sample and free androgen index was derived.

Results: Women with irregular cycles (n=41, 11%) had higher SOS and QUI (median difference 6 m/s and 7.2% respectively, $p<0.05$) and a trend to higher BUA (5.1 dB/MHz , $p=0.10$). These associations persisted after adjustment for ambient temperature, age, BMI and smoking. Total testosterone, free androgen index and SHBG were associated with all QUS measures (testosterone and FAI r +0.11 to +0.21, all $p<0.05$; SHBG r -0.14 to -0.16, all $p<0.05$) and the associations remained significant after adjustment. However, the associations between menstrual irregularity and QUS were partially attenuated after adjustment for hormonal factors especially FAI becoming non significant at all sites.

Conclusion: In our increasingly obesogenic society, irregular cycles are now associated with higher bone mass in a population-based sample of premenopausal women. The association between menstrual irregularity and bone mass is, at least, partially mediated by free testosterone while total testosterone, FAI, and SHBG are independently associated with bone mass.

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ALLELIC VARIATIONS OF RANK/RANKL/OPG SIGNALING SYSTEM IS RELATED TO BONE MINERAL DENSITY AND IN VIVO GENE EXPRESSION.

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Context: RANK/RANKL/OPG signaling system plays a crucial role in the regulation of bone resorption. Polymorphic variations in the genes of these crucial compounds may have an influence on bone metabolism.

Objective: In present study, we aimed to investigate the influence of OPG/RANKL/RANK allelic variations on the in vivo human gene expression of these genes, bone mineral density (BMD) and fracture incidence in Hungarian postmenopausal women.

Subjects and Methods: 360 osteoporotic patient (61.6 ± 7.9 years) was genotyped. All together, 8 single nucleotide polymorphisms (SNP) in the 3 genes have been investigated.

In addition, bone samples from 17 examined subjects were acquired for gene expression studies. Bone densities and fracture data have also been collected.

Results: All 3 SNPs in OPG and 2 SNPs in RANKL genes showed a strong correlation with BMD, however, the RANK SNPs had no such relationship. Haplotype analysis of these genes gave similar results. The ‘CCT' haplotype of RANKL promoter region, that was associated with decreased BMD, exhibited a significantly up-regulated expression of RANKL mRNA, while the other haplotypes of RANKL or OPG/RANK genes did not. In an “in silico” analysis, we could identify 7 transcription binding sites in the promoter of the ‘CCT', whereas only 2 such sites were found in case of other common ‘TTC' haplotype. No correlation between genetic variations and fracture data was found.

Conclusion: We have demonstrated a strong correlation between RANKL and OPG haplotypes and BMD as well as RANKL haplotypes and in vivo RANKL expression in Hungarian postmenopausal population.

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CONTRIBUTION OF FAT MASS AND OBESITY-ASSOCIATED (FTO) GENE TO BONE LOSS: THE DUBBO OSTEOPOROSIS EPIDEMIOLOGY STUDY

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Individuals with excessive bone loss are at increased risk of fracture. Common variants in the Fat mass and Obesity associated (FTO) gene are associated with body weight which is closely correlated with bone mineral density (BMD). In this study, we examined the relationship between the FTO gene and bone loss in postmenopausal women.

Bone mineral density at the femoral neck and lumbar spine was measured by dual-energy X-ray absorptiometry (DXA) at baseline and subsequent visits between 1989 and 2008 in 1142 women aged 60 years or above as at 1989 who were participants of the Dubbo Osteoporosis Epidemiology Study. Genotypes of the SNP rs9930506 in the first intron of the FTO gene were determined by a pre-design Taqman assay. The genotypic distribution was consistent with Hardy-Weinberg disequilibrium law with frequencies: 362 AA (31.7%), 565 AG (49.5%) and 215 GG (18.8%).

At baseline, there was no significant difference in femoral neck or lumbar spine BMD between the genotypes. However, the rate of BMD loss at the femoral neck was greater ($p = 0.03$) in women with the AA (-1.37 ± 2.80%/year) and AG genotype (-1.56 ± 2.46%/year) than in those with the GG genotype (-0.91 ± 2.90%/year). The effect remained statistically significant after adjusting for age or body weight. The same trend was observed for lumbar spine BMD, but the difference was not statistically significant ($p = 0.4$). The proportion of variance in femoral neck bone loss that could be attributed to the A allele at the FTO gene was 34%.

These results suggested that in postmenopausal women, the common variation at the FTO gene is associated with femoral neck bone loss with modest magnitude of association.

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EFFECTS OF TETRANDRINE ON THE NERVE CELLS' APOPTOSIS AND THE EXPRESSION OF BCL-2 AND BAX AFTER SPINAL CORD INJURY

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Objective To observe the expression of apoptosis factor, the mechanism of neuronal apoptosis and prefective effect of Tetrandrine(Tet) after acute spinal cord injury(ASCI) in SD rats.

Methods 95 Adult SD rats were divided at random into four groups: control group (5), SCI group (30), treatment group A (Tet group , 30), treatment group B (methylprednisolone group 30). The animal SCI model was established with Allen's method. The rats in control group were killed at 3 weeks later . The rats of the other three groups were sacrificed at 8 hours and 1,3,5,7,14,21 days after surgery ,then the spinal cord tissue of 5mm long were taken out .One part sections was stained with hematoxylin and eosin and the injuryed spinal cord tissue was observed. The expressions of apoptosis factors (bal-2,bax) were tested with immunocytochemistry technique., another part was put into the flow cytometry (FCM) .

Results The number of Bax positive cells in SCI group was more than that in group A, and group A was more than group B. But the number of bcl-2 positive cells in group B was more than that in group A , and group A was more than that in group B. The expressions of bcl-2 and bax genes reached the top at 7 days later in all groups .The number of apoptosis neurons in SCI group was more than that in group A, and group A was more than that in group B. The number reached the top at 7 days later.

conclusion Apoptosis was the main pattern by which the nerve cells died in ASCI . The apoptosis control genes bcl-2 and bax played important roles. Tetrandrine could inhibit apoptosis through inhibiting the expression of bax and promoting the expression of bcl-2 after ASCI in rats.

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EVALUATION OF SERVICE DELIVERY OF THE RURAL SOUTH AUSTRALIAN MOBILE BONE DENSITY SERVICE - THE PATIENT'S PERSPECTIVE.

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We have previously examined population and density statistics of subjects attending our rural DXA service. We have now surveyed attitudes to the service and willingness to travel for the test.

1,000 consecutive patients attending the Mobile Bone Density Service (MBDS) completed a questionnaire querying age, pension concession status, post code, how far they were prepared to travel for a bone density test and awareness of the Government Patient Assistance Transport Scheme (PATS - A scheme to make specialist medical services more accessible to rural South Australians by assisting with the cost of transport over 100km).

Patients were also asked if they had any suggestions on improving our service delivery.

Results: The highest proportion of patients were between 55 and 65 (32%). Males comprised 9.2% of all respondents.

Travel intentions showed that 42% would not travel to Adelaide for their study, with a further 11% not indicating any preference. Of the remainder, 49% indicated they would use the PATS scheme for their travel, with the older age groups indicating a preference for this option.

Overall patients were satisfied with the MBDS and very appreciative of the service provided to their community. No additional constructive comments were received.

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BMP/WNT ANTAGONISTS ARE UPREGULATED BY DEXAMETHASONE IN OSTEOBLASTS AND REVERSED BY ALENDRONATE AND PTH: POTENTIAL THERAPEUTIC TARGETS FOR GLUCOCORTICOID-INDUCED OSTEOPOROSIS

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Glucocorticoid-induced osteoporosis (GIO) is known to be caused by suppression of osteoblast-mediated osteogenesis, and is effectively treated by bisphosphonate and parathyroid hormone (PTH). However, the exact mechanisms by which glucocorticoid suppresses osteoblast function, or alendronate or PTH alleviate GIO are still unclear. We used osteoblastic MC3T3-E1 cells in order to clarify these mechanisms. Dexamethasone (Dex) (10^{-9}–10^{-7} M) strongly and dose-dependently suppressed mineralization of the cells. On the other hand, Dex (10^{-7} M) increased mRNA expression of bone morphogenetic protein (BMP) antagonists, follistatin and Dan, and mRNA expression of a Wnt antagonist, secreted frizzled-related protein-1 (sFRP-1) and a Wnt signal inhibitor, axin-2, while concomitantly decreased the expression of Runx2 mRNA and beta-catenin protein, downstream of the BMP and Wnt signal pathways, respectively. Moreover, pretreatments with alendronate (10^{-8} M) or human PTH (1-34) (10^{-8} M) totally or partially antagonized not only the Dex-induced enhancement in mRNA expression of follistatin/Dan and sFRP-1/axin-2 but also the Dex-induced reduction in Runx2 mRNA expression and mineralization. The present study shows that Dex suppresses the Wnt and BMP pathways by enhancing the expression of BMP and Wnt antagonists, suggesting that glucocorticoids can modify osteoblastic differentiation and activity through these pathways. The effect of bisphosphonate and PTH on the cancellation of these processes may partly explain their anti-GIO pharmacologic efficacy.

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AGE-RELATED BONE LOSS: THE EFFECT OF NEGLECTING INTRACORTICAL POROSITY

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Bone remodelling is surface dependent. While bone loss on trabecular and endocortical surfaces has been studied, intracortical remodelling has been given little attention. We have reported that intracortical remodelling accounts for a large proportion of bone lost with age and produced cortical thinning from 'within' as the cortex adjacent to the marrow

becomes trabecularized by coalescence of intracortical pores. The remaining cortex that appears compact is thinned and more porous while the more porous cortex adjacent to the marrow appears like 'trabecular' bone. We propose these two effects produce serious errors in the assessment of age-related structural decay in cortical and trabecular compartments. First, cortical porosity is calculated from the remaining compact cortex ignoring the porosity producing trabecularization and so underestimating it. Second, the fragments of the trabecularized cortex are measured as trabecular bone resulting in understimation of trabecular bone loss. To quantify the errors produced in estimating age-related bone loss we studied HR-pQCT in 121 women (aged 21 to 99) using HR-pQCT, and 22 specimens cadavers age 29 to 90 using scanning electron microscopy (SEM). Cortical porosity was directly measured including and excluding the porosity producing the trabecularized cortex; cortical density (CoD) and trabecular density (TrabD) were estimated including and excluding the trabecularized cortex. In vivo, in the 121 women, between 50 and 80 years of age, CoD appeared to diminish by 17% (from 892.8 to 792 mgHA/cc) when porosity producing the trabecularized cortex was ignored. When included CoD decreased by 51% (from 892.8 to 438mgHA/cc). When fragments of the trabecularized cortex were regarded as trabeculae TrabD diminish by 20% (from 148 to 117mgHA/cc). Removing the spurious effect of trabecularization, the diminution in TrabD was 3 times higher (66%, from 148 to 54mgHA/cc). Similar observations were made using SEM. Excluding porosity that produced the trabecularized cortex underestimated of porosity by up to ~2.5 folds (8 vs 20%). Reliable assessment of structural decay requires understanding and taking into account the effect of intracortical porosity on bone microstructure.

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NO ASSOCIATION BETWEEN POLYMORPHISMS OF PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA GENE AND PEAK BMD VARIATION IN CHINESE NUCLEAR FAMILIES

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Introduction: Proliferator-activated receptor-gamma (PPARG) gene is one of three PPAR nuclear receptors and while expressed, it is primarily found in white adipose tissue. Recent evidences have suggested that PPARG plays a critical role in osteogenesis.

Methods: We performed to genotype ten tagging SNPs (rs2972164, rs17036188, rs11128597, rs12636454, rs1801282, rs17817276, rs4135275, rs1151999, rs1175542, and rs3856806) in PPARG by TaqMan, and further test whether SNPs were associated with peak bone mineral density (BMD) variation at the spine and femoral neck of 401 Chinese nuclear families using quantitative transmissing disequilibrium test (QTDT). Furthermore, the association between SNPs in PPARG and BMD in 710 postmenopausal Chinese women was investigated.

Results: Using QTDT, for the population stratification, total family association, and within-family association, we failed to find that single-SNP and haplotype was significant associated with peak BMD at lumbar spine and femoral neck. Subsequent permutations were in agreement with these findings. Meanwhile, we found that only rs1801282 was significantly associated with BMD at spine in postmenopausal women (P=0.013), and subjects with CC genotype had higher BMD in spine. In addition, no significant association was found between single-SNP and haplotype and BMI either in premenopausal or in postmenopausal women.

Conclusions: Although confirmation of our findings is required in other population, the genetics polymorphisms in PPARG is not a contributor to the observed variability in peak BMD in Chinese women.

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QUANTITATIVE ULTRASOUND MEASUREMENTS PREDICT FRACTURE IN OLDER WOMEN: A 10-YEAR LONGITUDINAL STUDY

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Although it is known that heel quantitative ultrasound (QUS) is correlated to DXA bone mineral density, there are limited longitudinal data on the association between QUS measurements and fracture risk.

This study examines the association between heel QUS measurements and incident osteoporotic fracture in a cohort of 1500 women aged 70-85 years when recruited in 1998 from the population. After finishing a five year RCT of calcium supplementation (CAIFOS), they were then recruited into a five year epidemiology study, the CARE study. Calcaneal QUS measurements of the left foot including broadband ultrasound attenuation (BUA), speed of sound (SOS) and stiffness were obtained using an ultrasound densitometer (Lunar Achilles) at baseline. Clinical incident osteoporotic fractures, excluding face and digits were confirmed from radiographic report. Totally 1436 women with baseline QUS measures were included in this analysis.

Baseline age, BUA, SOS and stiffness were 75.2 ± 2.7 years, 100.4 ± 7.9 dB/MHz, 1512.6 ± 25.8 m/s and 70.4 ± 11.5%, respectively. Over the study, 262 subjects had incident non-vertebral fracture, 94 subjects had incident clinical vertebral fracture and 324 subjects had at least one incident fracture at any site. Cox's proportional hazards analyses adjusted for baseline age and weight, previous fracture and calcium treatment group showed that one SD decrease in BUA, SOS or stiffness was associated with 36-42%, 43-59% and 40-45% increase in the risk of non-vertebral fracture, vertebral fracture and any fracture, respectively.

	Non-vertebral fracture hazard ratio (95%CI)	Vertebral fracture hazard ratio (95%CI)	All fracture hazard ratio(95%CI)
BUA (1SD decrease)	1.414 (1.236-1.616)	1.427 (1.135-1.792)	1.425 (1.263-1.610)
SOS (1SD decrease)	1.359 (1.193-1.548)	1.592 (1.267-2.004)	1.404 (1.248-1.582)
Stiffness (1SD decrease)	1.418 (1.244-1.618)	1.563 (1.245-1.957)	1.451 (1.289-1.634)

The results show that lower heel QUS measures are associated with a higher risk of incident fracture. QUS is a useful tool for identifying those with high fracture risk.

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VITAMIN B12 AND ITS LINK TO BONE HEALTH IN THE MALE POPULATION

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Introduction: Vitamin B_{12} deficiency has been implicated in the pathogenesis of osteoporosis as it leads to increased homocysteine levels and potentially decreased bone density.

The purpose of this study was to determine whether there was a correlation between serum vitamin B_{12} deficiency and diminished bone health in men.

Methods : We analysed sera from 1145 males representative of the Geelong population for vitamin B_{12}, type I collagen C-telopeptide (CTx), procollagen type 1 N-terminal propeptide (P1NP) and 25-hydroxyvitamin D_3 (25-OHD_3) using the automated Roche Modular Analytics E170 analyser.

Subjects were divided into two groups based on their vitamin B_{12} status (vitamin B_{12} deficient defined as <150pmol/L). Results were analysed using a commercial statistics program (SAS version 9.1).

Results: Vitamin B_{12} deficiency was present in 61 men (mean age = 68 years, sufficient vitamin B_{12} mean age = 60 years). P1NP levels were lower in vitamin B_{12} deficient men (P1NP median= 35 ± 3 µg/L) compared to men with normal vitamin B_{12} levels (P1NP median= 43 ± 1 µg/L, p=0.002).

CTx levels were not different between subjects that were vitamin B_{12} deficient and those with normal vitamin B_{12} levels (p=0.401).

A significant linear association was observed between vitamin B_{12} and 25-OHD_3 concentrations (r=0.066, p=0.025).

Bone mineral density (BMD) measured at Wards triangle (WT) was lower in the vitamin B_{12} deficient subjects (BMD mean= 0.756 ± 0.019, n=55) than in vitamin B_{12} sufficient men (BMD mean= 0.791 ± 0.005, n=1048, p=0.039). Spine, femoral neck and trochanter BMD readings showed no significant differences (p=0.712, p=0.174, and p=0.437 respectively).

Conclusion: While results of our study may indicate a weak association between vitamin B_{12} deficiency in males and reduced bone formation this could possibly be attributed to age effects. There appears to be no effect on bone resorption.

Interestingly, the link between vitamin B_{12} and bone metabolism is supported by the significant association that was found between vitamin B_{12} levels and WT BMD.

Ultimately, more studies are needed to determine if vitamin B_{12} deficiency has an adverse affect on bone health in the male population.

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COMPARISON BETWEEN HIP GEOMETRY IN US CAUCASIAN AND CHINESE WOMEN

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Hip geometry has often been pointed to as a possible contributing factor in relative fracture risk and as a source of difference in hip fracture risk reported in Caucasian and Asian populations. The current study compares factors related to hip geometry in these populations by evaluating hip geometry in adult Caucasian women in the USA and Asian women in China.

Fifty US Caucasian women and fifty Chinese women between 30 and 45 years of age were examined on two Norland XR-46 scanners by experienced operators. These scans were audited for good quality by the coauthors (US Caucasian by TVS and Chinese by JMW). A routine Hip Scan was done to assess bone density at the Femur Neck, Trochanter and Ward's regions. Operator defined regions analyzed the Upper Neck, Lower Neck and Femur Shaft bone density and the Norland Ruler Tool was used to measure the Femur Neck Length. Analysis of differences between the Caucasian and Chinese populations was by comparing population statistics and regression analysis.

Examining results of these studies showed Asians have a significantly shorter Femur Neck Length than the Caucasians (57.9mm vs 60.6mm, $p<0.0001$). While t-test did not show significant differences between Caucasian and Asian Femur Neck, Trochanter, Ward's, Upper Neck or Lower Neck bone density, there was a tendency to a slightly greater Femur Shaft bone density in the Asian than in the Caucasian women ($p<0.088$). ANOVA analysis between Femur Neck and other parameters showed no difference in slope or intercept regressions with the Trochanter and Ward's regional density. While no differences were seen in the slope, ANOVA analysis showed significant differences in the intercept when doing regressions between bone density in the Femur Neck and Upper Neck ($p<0.001$), Lower Neck ($p<0.005$) or Femur Shaft (($p<0.0001$).

In conclusion, a shorter Femur Neck in the Asian women with a relative difference in Upper Neck, Lower Neck and Femur Shaft bone density is indicated. Hip geometry may be reacting to mechanical forces and may ultimately affect hip fracture differences in the Asian and Caucasian populations.

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DRINK PROTECTS BMD AND DECREASES FRACTURE RISK IN ELDLY

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BACKGROUND: Studies on relationship between alcohol intake and bone mineral density (BMD) in older people have shown mixed results.

OBJECTIVE: To study the association between alcohol intake and BMD, falls risk and fracture in men and women aged 50-80 years.

DESIGN: A total of 862 randomly selected subjects (mean 63 years, range 51-81, 51% male) were studied at baseline and 2.6 years later. BMD at lumbar spine and hip of participants was assessed by DXA. Alcohol intake was assessed by food-frequency questionnaire. Physical activity was collected by questionnaire and pedometer. Falls risk (Z score) was determined using the short form Physiological Profile Assessment (PPA). Data on prescription medication and incident fracture was collected between baseline and follow up.

RESULTS: Alcohol intake in males was higher than in females (20.9g/d vs 9.8g/d, $P<0.05$). Alcohol intake was positively associated with change in male BMD at the lumbar spine ($\beta=0.02\%$ per gram alcohol, $P<0.05$) and hip ($\beta=0.02\%$, $P<0.05$) after adjusting for age, BMI, medication and physical activity. Similarly, number of drinks of alcohol was positively associated with change in male BMD at lumbar spine ($\beta=0.15\%$ per glass, $P<0.05$). With mixed model analysis we found that drinks in male increased hip BMD at 0.09 mg/cm2 /gram alcohol per day/year or 0.76 mg/cm2 /glass of alcohol per day/ year ($P<0.05$) . Alcohol intake (per glass) in females was not associated with BMD but decreased non-vertebral fracture risk (RR=0.73, 95%CI 0.55-0.98). There was no association with fracture in males (RR=1.02, 95%CI 0.84-1.24). No significant relationship was found between alcohol and falls risk.

CONCLUSION: Alcohol intake may prevent hip BMD loss in older men and protect women from non-vertebrae fracture. Longer term follow up will required to confirm these associations.

Category 4. Clinical Disorders other than Osteoporosis

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CELLULAR MORPHOLOGY OF CULTURED PAGETIC OSTEOBLASTS

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Paget's disease of bone is characterised by focal areas of disorganised bone architecture resulting from abnormal and increased bone turnover. In the lytic phase of Paget's disease the overactive pagetic osteoclast is thought to commence the cycle of abnormal bone turnover by increasing bone resorption which is then followed by concomitant increased bone formation by surrounding osteoblasts (sclerotic phase). The initiating microenvironment and factors involved in the development of this disease are still unknown. It is well established that during normal bone remodelling osteoblasts regulate osteoclast differentiation and activity. For this reason we have studied the pagetic osteoblast and its potential role in the development of Paget's disease. A number of genes were found to have differential expression when pagetic osteoblasts were compared to osteoblasts cultured from healthy bone (1). To further investigate pagetic osteoblasts, transmission electron microscopy (TEM) was used to examine the ultrastructure of these bone cells.

Trabecular bone explants from seven individuals with Paget's disease and three individuals without Paget's disease were collected during joint replacement surgery. All pagetic bone samples were confirmed to be from pagetic sites by x-ray or scintigraphy. Primary osteoblast outgrowth cultures from pagetic and non-pagetic trabecular bone explants were grown in culture flasks until near confluency. Following trypsinisation, isolated osteoblasts were cultured on plastic discs for several days and these osteoblast covered discs were then processed for examination by TEM following standard procedures. Several individual osteoblasts were examined from each of the 10 patients on a Tecnai™ G² Spirit TWIN transmission electron microscope. Non-pagetic osteoblasts displayed typical and expected ultrastructural features. In comparison, pagetic osteoblasts contained extensive rough endoplasmic reticulum (RER) in long multiple and parallel arrays, and vesicles were frequently larger and more abundant.

These morphological changes, taken together with the demonstration of changes in gene expression in pagetic osteoblasts, suggest that these cells may be contributing to the pathogenesis of Paget's disease.

(1) Naot D, et al. JBMR 2007; 22:298-309

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ASSESSMENT OF 3D BONE MICROARCHITECTURE IN FEMORAL NECK FROM POSTMENOPAUSAL WOMEN WITH HIP FRACTURE OR HIP OSTEOARTHRITIS

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In contrast to osteoporotic patient (OP), patient with hip osteoarthritis (OA) may be protected against hip fractures, which were attributed to a cortical thinning along the inferoanterior-superoposterior axis of the femoral neck (1). Moreover, we have previously reported an alteration of trabecular bone in OP compared to OA patients by histomorphometry (2).

To assess the difference of hip fragility in OA and OP, we compared the distribution of cortical and trabecular bone in the ultradistal femoral neck samples (~3mm thick) obtained after total hip replacement in 21 OA (66 ± 8 yrs) and 18 OP (79 ± 8 yrs) menopausal women by HR-pQCT: a direct 3D evaluation method (XtremeCT, Scanco Medical AG) and by histomorphometry, performed and averaged on three 10µm-thick sections spaced of 800µm. High correlations were observed between both techniques for trabecular bone volume, number, thickness, separation and cortical thickness ($0.51<r<0.81$, $p<0.01$). The connectivity is also highly correlated ($r=0.57$, $p<0.001$) between both techniques, as well as trabecular bone pattern factor measured in 2D with the structural model index (SMI) measured in 3D ($r=0.61$, $p<0.001$).

3D measurements showed that trabecular bone volume was 43% lower in OP than OA ($p<0.01$), associated with a loss of the trabecular connectivity (-50%, $p<0.01$) and a more rod-like structure (SMI, 22%, $p<0.01$) mainly in the inferior (34%, $p<0.01$) and posterior (22%, $p<0.05$) quadrants. Cortical thickness was found to be lower in the inferior and

posterior quadrants in OP than in OA (respectively -13% and -21%, $p<0.05$), but it was still the highest in the inferior quadrant in both groups.

In conclusion, 3D methods confirmed the alteration of trabecular and cortical bone found by histomorphometry in OP when compared to OA and in addition showed the prevalence of the rod-like structure in OP. These results strongly suggest that hip fragility results not only from the cortical but also from the trabecular bone deterioration.

(1) Bell KL, Loveridge N, Power J, Garrahan N, et al. Structure of the femoral neck in hip fracture: cortical bone loss in the inferoanterior to superoposterior axis. J Bone Miner Res 1999;14:111–9.

(2) Blain H, Chavassieux P, Portero-Muzy N, Bonnel F, et al. Cortical and trabecular bone distribution in the femoral neck in osteoporosis and osteoarthritis, Bone 2008;43:862-8.

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PROGRESSION OF SKELETAL DISEASE IN MPS DISORDERS.

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The mucopolysaccharidoses (MPS) are a group of related inborn metabolic diseases that arise from impaired turnover of extracellular matrix glycosaminoglycans. A progressive skeletal dysplasia termed dysostosis multiplex is a cardinal feature of six of the eleven MPS disorders. This study was undertaken to characterise MPS bone development from birth to skeletal maturity in order to provide a framework for potential therapeutic intervention points. A histomorphometric analysis was carried out in the MPS VI cat vertebrae using an automated image analysis system (Quantimet 520, version 4.0, Cambridge Instruments, Cambridge, UK). Fluorochrome dyes were administered prior to sacrifice to measure bone formation rate and mineral apposition rate. At birth bone mineral volume in the MPS VI vertebrae fell within the normal range (BV/TV = 15.3 ± 3% and 19.5 ± 5% respectively), as did bone mineral surface, trabecular number, thickness ad specing. In the 3 weeks after birth BV/TV decreased in both normal and MPS VI vertebrae via a decrease in trabecular number. With increasing age BV/TV in the normal vertebrae increased to a level commensurate with that observed at birth (19.3 ± 4%) via an increase in trabeculae thickness. In contrast, BV/TV in the MPS VI vertebrae remained low attaining a maximum value of 2.4 ± 1% at skeletal maturity (11 months of age). Although a small increase in trabeculae thickness was observed with increasing age in MPS VI, this did not match the increase observed in normal and was offset by a continual loss of trabecular number. Bone formation rate was highest in younger animals and decreased with age in both normal and MPS VI vertebrae although the values for MPS VI were consistently lower than normal at all ages analysed. Mineral apposition rate in MPS VI, however, was normal. The observation that bone content and structure has not deviated significantly from normal at birth is encouraging from a therapy point of view. Early treatment and strategies to support osteoblast function via the efficient delivery of replacement enzyme or adjunct therapies should improve skeletal outcome for MPS children.

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PAGET'S DISEASE OF BONE – BECOMING A RARITY?

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Paget's disease is a chronic bone disorder of unknown cause. Current theories of its aetiology have focussed on recently discovered disease-associated genes. However, an apparent reduction in both prevalence and disease severity in recent decades is in conflict with the genetic hypothesis. We undertook a radiographic survey to determine the current prevalence of Paget's disease in New Zealand (previously recognised as a high prevalence area). 3350 plain abdominal radiographs taken in 2005-6 in subjects of European descent ≥55 years old were examined for the characteristic signs of Paget's disease. The results were compared to those of a similar survey from 1996-8 (1). The medical record of affected subjects was examined to determine when the disease had been first recognised and the plasma alkaline phosphatase (ALP; normal <120u/l) at the time of first diagnosis.

Paget's disease was detected in 87 radiographs (2.6%). In 55 cases (63%) it was already known to have been present, for a mean 14 years beforehand. At diagnosis the newly recognised or "incident" cases were significantly older (mean

age 86 vs 67 yrs, p<0.0001) and had milder disease (geometric mean ALP 139 vs 239 u/l, p<0.0001) than the known cases. Compared to the 1996-8 survey, the age distribution of affected patients was shifted to the right, with a significantly lower proportion in the youngest age group (55-69 years, p<0.004). These results confirm the secular trend of Paget's disease presenting later in life and in milder form, strongly suggesting that there are important environmental determinants. There are relatively few incident cases and these are mostly in the very elderly. Given the secular trend and limitations to life expectancy, it is predicted that Paget's disease will become increasingly rare.

(1) T Doyle et al, Bone 31: 616– 9; 2002.

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KNEE AND HIP RADIOGRAPHIC OSTEOARTHRITIS PREDICT TOTAL HIP BONE LOSS AND HIP FRACTURE IN OLDER ADULTS

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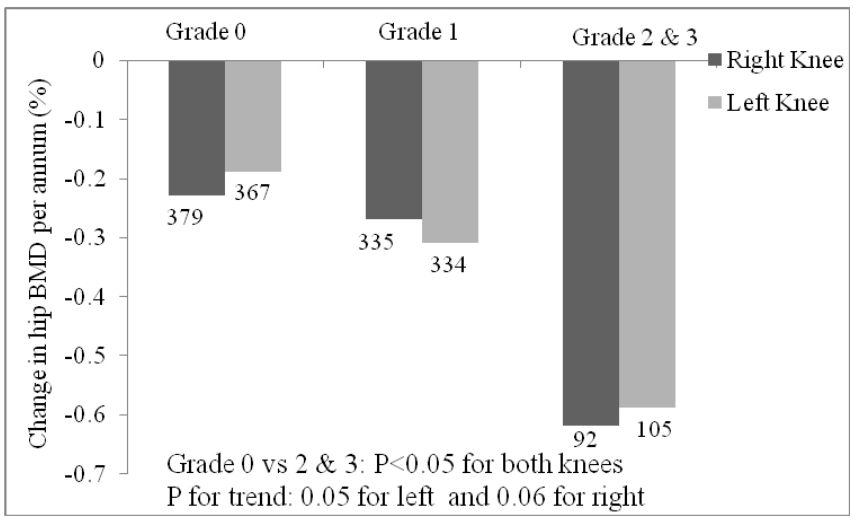
Background. The relationship between osteoarthritis (OA) and osteoporosis and subsequent fracture remains controversial, even given that the first study indicating an inverse relationship between these two common age-related skeletal disorders was reported three decades ago.

Objective. To determine the association between hip and knee radiographic OA, change in total hip bone mineral density (BMD) and fractures over 2 .6 years.

Methods. A total of 867 randomly selected subjects (mean 62 years, range 51-80, and 49% female) were included. Hip and knee joint space narrowing (JSN) (0-3) and osteophytes (0-3) in both lower limbs was assessed using Altman's atlas. Total hip BMD was measured by DXA and total fractures were recorded.

Results. Radiographic OA (score of JSN or osteophytes > 0) was common in this sample (hip: 45%, knee: 68%). In multivariable analyses, percentage change in total hip BMD per annum was predicted by right and left hip axial JSN (grade ≥2 vs 0: β = - 0.69% to - 0.80%, both P<0.05), right hip superior femoral osteophytes (P<0.05), right and left medial tibiofemoral JSN (grade ≥2 vs 0: β = - 0.35% to -0.37%, both P<0.05) and osteophytes (both P<0.05), independent of each other and joint pain. Grade 1 hip axial JSN and knee medial JSN were insignificantly associated with increased loss of total hip BMD. Incident hip fracture (0.7%) was significantly predicted by left hip superior JSN, right knee lateral JSN and tibial osteophytes but these became non-significant after adjustment for change in total hip BMD.



Conclusions. Older subjects with radiographic hip and knee OA have higher total hip bone loss and hip fracture risk over 2.6 years regardless of symptoms. Prevention of hip bone loss and fracture should be considered in those with radiographic hip and knee OA.

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BREAST FEEDING DOES NOT PROTECT AGAINST URINARY TRACT INFECTION IN THE FIRST 3 MONTHS OF LIFE, BUT VITAMIN D SUPPLEMENTATION INCREASES THE RISK BY 76%

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Urinary tract infections (UTI) are the most common serious illness in infants. Breastfeeding has been reported to protect infants from UTI, while hypervitaminosis D can cause UTI. To further investigate factors influencing UTI rates, we performed a chart review of infants 0-3 months of age who had urine cultures obtained at Ochsner Clinic Foundation, New Orleans, LA, between 2001-2006. 40% of the children who had urine cultures were breastfed, and 18.7 percent of the children were exclusively breastfed, similar to previously reported rates at our hospital. 20% of all of the urine cultures tested positive. As expected, a greater percentage of urine cultures in females were positive (22.5%) vs. those obtained from males (18.1%, $p<0.05$). However, the rates of positive urine cultures in exclusively breastfed children (22%) did not differ from those of formula fed infants (21%, NS). The relative risk of UTI with breastfeeding versus formula feeding was 1.03 (0.58-1.82) and the relative risk of UTI with any breastfeeding versus no breastfeeding was 0.92 (0.58-1.45). We thus found no evidence that breastfeeding protects against UTI. We were concerned that a greater percentage of breastfed infants may have received vitamin D supplementation, which may increase the rates of UTI in this group and confound our analysis. Vitamin D supplementation did significantly increased the risk of UTI, with a relative risk of 1.76 (1.07-2.91, $p<0.05$). However, vitamin supplementation rates were low (<15 %) and did not differ between the groups. Furthermore, formula fed infants showed the greatest increased risk of UTI with vitamin D supplementation (RR=2.24 (1.29=3.90), $p<0.05$); neither exclusively breastfed infants (RR=1.61(0.46-5.59), NS) nor infants receiving any breast milk (RR=1.12(0.38-3.28), NS) showed significantly increased UTI risk with vitamin D supplementation. Overall, our data indicate that breastfeeding does not protect from UTI, but supplementing infants with vitamin D increases the risk of UTI by 76%, the greatest risk being seen in formula fed infants. We would therefore recommend that parents whose children are formula fed be advised against providing vitamin D supplements to their children, and breastfed children be supplemented with caution.

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DESIGN OF AN INTERNATIONAL, RANDOMISED TRIAL OF GENETIC TESTING AND TARGETED ZOLEDRONIC ACID THERAPY TO PREVENT SQSTM1 MEDIATED PAGET'S DISEASE: THE ZIPP TRIAL

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Background: Paget's disease of bone (PDB) is characterised by increased bone turnover affecting one or multiple bones throughout the skeleton. Genetic factors play an important role in the disease and mutations of the SQSTM1 gene have recently been found to be an important cause occurring in between 20% - 50% of patients with a positive family history. Bisphosphonates are highly effective at suppressing elevated bone turnover which is characteristic of PDB and can help bone pain, but they are of limited benefit in patients with established disease who have already developed complications.

Trial objective: In this study, we will test the hypothesis that genetic testing coupled with prophylactic treatment with Zoledronic acid can prevent the development of raised bone turnover and focal bone lesions in carriers of SQSTM1 mutations.

Trial design: Subjects > 30 years, who have a positive family history of PDB but who have not yet developed clinical signs of PDB, will be screened for the presence of SQSTM1 mutations. Those who are found to carry SQSTM1 mutations will be invited to take part in an intervention study. They will be randomised into either a biochemical marker sub-study or a bone-lesion sub-study depending on different stratification and inclusion criteria.

In both sub-studies, participants will receive an infusion of placebo or Zoledronic acid. Repeat infusions will be given after 30 months. Participants will be followed up annually for 5 years and at each visit biochemical markers of bone turnover will be studied. The development of new bone lesions will be assessed by radionuclide bone scan after 5 years. The effects of the intervention on bone pain and quality of life will also be studied.

People who do not have the SQSTM1 mutation will also be invited to take part in an observational sub-study to evaluate the effects of genetic testing on quality of life and anxiety.

Results: It is hoped that the results of this study may underpin the introduction of a programme of genetic testing and targeted intervention for familial PDB in routine clinical practice improving the outlook for people who have a family history of the disease.

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SUBTROCHANTERIC AND SHAFT OF FEMUR FRACTURES AUDIT 2003 TO 2008

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A total of 600 patients, both males and females were identified from hospital records who were admitted with the above fractures. Those excluded were motor vehicle accidents (17.9%), intratrochanteric fractures (5.4%) and tumours (0%). Patients who were less than 20 were also excluded (25%). Seventy two percent (72%) of these patients were female and 28% were male. The majority (89%) were over 60 with a large percentage (51%) over the age of 80.

Ninety three percent (93%) had simple transverse or short oblique fractures and 13.8% had comminuted in the form of a medial or a lateral wedge fractures on xray review. On admission only 13.8% were on Calcium, 6.9% on Vitamin D, 13.8% on steroids and 20.7% on Alendronate. There were none on Etidronate and only 3.4% were on Methotrexate or Cytotoxic agents. Almost all of these people lived at home, had no prior bone density scan and 65.5% had a prior fracture.

Subtrochanteric fractures are uncommon fractures, most occur in females who have had prior fractures. No one appeared to have had previous investigations for osteoporosis and only 11.4% were on treatment for osteoporosis. Approximately 20% of these people however, appeared to be on Bisphosphonates. The significance of this is uncertain. Further data will be presented.

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BURN-INDUCED CHANGES IN THE ILEAL CALCIUM SENSING RECEPTOR AND STANNIOCALCIN

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Burn injury alters Ca metabolism leading to hypocalcemia, hypoparathyroidism, and hypercalciuria. These consequences make Ca less available to compensate for burn-associated bone loss due to endogenous glucocorticoids and inflammatory cytokines. We previously reported up-regulation of the parathyroid Ca sensing receptor (CaR) in burned sheep(Murphey et al *Crit Care Med* 2000) ; this finding could explain the above changes by reducing the set point for Ca suppression of parathyroid hormone (PTH) secretion. We also reported that CaR was distributed in the cardiac and arterial endothelium of sheep and remained unchanged after burns(Klein et al *Burns* 2008). *Hypothesis.* Burn injury will up-regulate the intestinal CaR to compensate for the hypocalcemia, maintain high intestinal Ca concentration and reduce cell damage. *Aim.* Determine the location and density of the CaR in ileum from burn-injured and sham-burned sheep. *Methods.* We used fluorescence deconvolution microscopy to obtain acquisitions of probed tissues from sham-burned and burned Merino sheep and formulated detailed images and models from stacked sections of ileum probed for the CaR. The samples were further probed for the presence of stanniocalcin-1 (STC-1), a 25-kDa

homodimeric glycoprotein hormone, which inhibits gut Ca absorption and enhances gut phosphate uptake. *Results.*Villous architecture remained intact. There was a threefold increase in CaR following burns vs sham, with localization to the microvasculature of the lamina propria (13165 vs 5571 pixels cross section, 18,105 vs 6066 pixels longitudinal section). In contrast, while CaR was also found in the vessels of the submucosa and adventitia, and the myenteric plexus (Auerbach's), it was unchanged following burns. STC-1 was also increased threefold but localized to the villous base, suggesting another role. There was little of no STC-1 in other areas, including vascular endothelium. *Conclusions.* CaR is an intrinsic component of ileum and is up-regulated by injury to compensate for pathologic ionic transport disruptions in order to maintain neuromuscular Ca signaling. STC-1 synthesis is induced with injury, suggesting a role in Ca transport protein regulation and phosphate-associated Ca absorption.

(1) Murphey E, Chattopadhyay N, Bai M et al, Crit Care Med 2000; 28: 3885-90
(2) Klein GL, Enkhbaatar P, Traber DL et al, Burns 2008; 34: 370-5

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DO OSTEOBLASTS CONTRIBUTE TO THE DEVELOPMENT OF PAGET'S DISEASE?

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Paget's disease is a common bone disease characterised by foci of accelerated bone turnover. While osteoclasts are often considered to drive the disease, bone formation is also abnormal suggesting primary or secondary osteoblast involvement. We have cultured osteoblasts and bone marrow from 23 patients with Paget's disease, and from non-pagetic bone from patients with and without the disease. RNA from these cells has been used to identify changes in gene expression between pagetic and non-pagetic cells using microarrays and real time PCR. Previously we reported that pagetic cells had an unchanged RANKL/OPG ratio, increased interleukin 6, Dkk1, and alkaline phosphatase, and decreased osteocalcin and bone sialoprotein expression. In the current study we used low density array-based real time PCR analysis to further investigate differential gene expression in pagetic osteoblasts and bone marrow cells.

mRNA levels of a number of genes with important roles in osteoblast differentiation and function were significantly down-regulated in the pagetic osteoblasts. These included the master regulator of bone formation, Runx2, and two additional transcription factors, Dlx5 and SATB2. Fibroblast growth factor receptor 2, which mediates signals that promote osteoblastic proliferation and differentiation was also down-regulated in pagetic osteoblasts. Two secreted proteins found to be down-regulated were BMP2, a potent stimulator of bone formation, and tenascin C, which influences cell morphology. Genes significantly upregulated in pagetic osteoblasts included matrix gla protein, an inhibitor of mineralisation which shows decreasing expression as osteoblast differentiation progresses, and the chemokine, monocyte chemotactic protein 1, which is induced by various factors including interleukin 6, and can stimulate monocyte recruitment and osteoclastogenesis .

Taken together with results from our previous studies, it appears that osteoblasts derived from pagetic lesions are at an earlier differentiation stage than osteoblasts from healthy bone and that they produce higher levels of local factors that increase osteoclastogenesis. The osteoblasts retain their abnormal phenotype despite being removed from the pagetic bone microenvironment for a number of weeks, suggesting that there is a primary abnormality in pagetic osteoblasts and they play an important role in the development of the pagetic lesion.

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SELECTIVE BONE MARROW TRANSPLANTATION USING LOW-DOSE CHEMOTHERAPY

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For genetic bone diseases such as *Osteogenesis Imperfecta* (OI) there is no curative treatment. Bone marrow transplantation (BMT) is an appealing therapeutic strategy as it has the potential to deliver healthy osteoprogenitors to affected patients. Donor cells may be allogenic, or autogenic following correction of the genetic defect using gene

therapy. Previous attempts to use BMT for OI have yielded inadequate outcomes, but have been associated with low levels of donor cell engraftment in bone.

We are exploring the potential of applying BMT in combination with *in vivo* selection for osteoprogenitors. We are adopting an approach where donor cells express a mutant version of the DNA repair enzyme O6-methylguanine methyltransferase (MGMT). This $MGMT^{P140K}$ mutant is resistant to the drug O^6-Benzylguanine (O^6BG), such that it is able to repair damage induced by the chemotherapeutic Carmustine (BCNU). This approach has been successfully used to select for haematopoietic progenitors *in vivo* in mouse models, and is currently undergoing human trials.

In a pilot study, whole marrow from transgenic $Mgmt^{P140K}$ (C57BL/6) donors were engrafted into Balb/c recipients at 2 months, and treated with or without selection over 4 months. The level of engraftment of haematopoietic donor cells was measured in peripheral blood mononuclear cell populations using FACS assay. One mouse that had not received selective chemotherapy (20% haematopoietic engraftment) and one mouse that had received chemotherapy (>90% engraftment) were selected for further analysis. Engraftment in the adherent marrow stromal cells (MSC) was measured using the same FACS assay. The untreated mouse possessed 6.2% donor MSCs, while the mouse that underwent selection had 44% donor MSCs. This demonstrates a correlation between haematopoietic engraftment and MSCs that we subsequently confirmed in additional n=10 mice using a semi-quantitative PCR approach (R^2=0.66).

These data illustrate that we can use *in vivo* chemo selection to substantially enrich for donor osteoprogenitors and support the continued investigation of BMT based strategies. We are currently carrying out a more detailed study tracking the distribution of donor MSCs throughout bone tissue with and without selection.

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GADOLINIUM-ENHANCED MRI EVALUATION OF THE VASCULAR INGROWTH INTO INTER-CONNECTED POROUS HYDROXYAPATITE CERAMIC GRAFTED IN JUXTA-ARTICULAR INTRAOSSEOUS CYSTIC LESIONS OF PATIENTS WITH RHEUMATOID ARTHRITIS

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[Background and Objectives] We had performed inter-connected porous hydroxyapatite (IP-CHA) (Stryker Co., Tokyo, Japan) grafting against juxta-articular intraosseous cystic lesions associated with impending pathological fracture involving the articular surface in patients with rheumatoid arthritis (RA). This new technique had yielded good results with implant incorporation into host bone and without peri- and post-operative fracture (1) . However, time required for bone conduction into grafted IP-CHA in patients with RA whose subchondral bone marrow is also involved by inflammation remains to be clarified. So objectives of this study are to clear the bone conductivity of grafted IP-CHA in RA patients.

[Methods] We evaluated the vascular ingrowth into IP-CHA grafted in five juxta-articular intraosseous cystic lesions i ncluding olecranon (n=2), proximal radius (n=1) and distal radius (n=2) in four RA patients. Diameter of IP-CHA was from 1 to 2 mm. Minimum and maximum diameters of cystic lesion were from 8 to 15 mm and from 10 to 19 mm, respectively. After curettage, complete resection of the capsule of the cyst and multiple drilling of the cyst wall, IP-CH A granules were grafted. Plain radiograph and gadolinium(Gd)-enhanced MRI were examined at one, three, six months after surgery.

[Results and Discussion] Sequential plain radiographs showed that the density of the implanted IP-CHA increased and the granules appeared to become fused and incorporated into the surrounding host bone until six month after surgery. Gd-enhanced MRI showed the ring enhancement in grafted IP-CHA at one month in all lesions and whole area enhancement at three month in all lesions. In the case of benign bone tumors, it is reported that bone forming area in grafted IP-CHA evaluated with bone scintigram was corresponded to vascular in-growing area evaluated with the Gd-enhanced MRI. So, these findings indicated the early bone conduction into IP-CHA even in lesion in RA patients.

[Summary] Our data showed that IP-CHA grafted into juxtaarticular cystic lesions sized under 20 mm diameter is fully vascularized within 3 months in patients with RA.

[Conclusion] Our findings suggested that bone conduction resulting from vascular ingrowth into grafted IP-CHA is not retarded and growing satisfactorily even in RA patients.

(1) Kuriyama K et al. Treatment of juxta-articular intraosseous cystic lesions in rheumatoid arthritis patients with interconnected porous calcium hydroxyapatite ceramic. Mod Rheumatol 2008, in press

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BONE HEALTH AND AGE OF COMMENCEMENT OF ANTI-EPILEPTIC MEDICATION: AN AED-DISCORDANT TWIN AND SIBLING PAIR STUDY

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AIM: To investigate:

(1) bone health in gender-matched, AED-discordant twin and sibling pairs; and

(2) associations of age of onset of epilepsy with bone mineral density (BMD), stratifying pairs where AED-user commenced AED before or after 18 years, an age where majority of bone mass is attained.

BACKGROUND: Patients taking anti-epileptic drugs (AEDs) have increased fracture risk. Data is limited regarding effects of age of commencement of AEDs, particularly with respect to potential effects AED-use at younger ages may have on peak bone mass.

METHOD: Fifty AED and epilepsy-discordant pairs were studied. BMD was measured (Hologic 4500A/1000W) at lumbar spine (LS), total hip (TH), femoral neck (FN), total forearm (FA) and total body bone mineral content (TBBMC). Data adjusted for age, height, weight. Paired t-tests calculated mean within-pair differences (MWPD). Independent t-tests compared within-pair differences of pairs where the AED-user commenced AED under vs. over 18 years.

RESULTS: 40 female, 10 male pairs (17 monozygous, 15 dizygous twins and 18 sib pairs), mean (SD) age 44.5 (15.8) years were studied.
Overall group: There was a significant MWPD (AED-user with lower results) in height, FN and LS, despite higher calcium intake.
In pairs where AED-user commenced AED before age 18y, the AED-user had significantly lower BMD at TH, FN and TBBMC than pairs where the AED-user commenced after 18y despite no significant difference in AED duration [Mean(SD) AED use: <18years: 20.5 (14.6)y; >18years 15.2 (12.1)y, p=0.170.]

CONCLUSION: AED-users have reduced BMD at clinically-relevant sites for fracture. Where AED was commenced before 18 years, effects at TH and FN are even more marked. Whether this is attributable to AED-associated reduction in peak bone mass or is influenced by duration of epilepsy and its therapy requires further, longitudinal study.

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A LONGITUDINAL STUDY OF BONE DENSITY IN REASSIGNED TRANSSEXUALS

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Sex hormones are required for the attainment of peak bone density during growth and its maintenance during adult life. If physiological withdrawal of these hormones occurs, osteoporosis may develop rapidly. Similarly, hormonally reassigned individuals may have potential for rapid bone loss when deprived of their phenotypic hormones.

In this study, a prospective collection of bone mineral density data in 185 transsexuals presenting for hormone reassignment was performed. On further assessment, two patients were concluded homosexual and seven categorized as gender of origin giving a final dataset of 176 individuals for analysis.

Dual energy X-ray (DXA) bone densitometry of the lumbar spine and femoral neck was performed serially with the longest follow up being 18 years.

Only a minority had measurements prior to any hormonal manipulation due to logistic constraints. DXA data were analyzed using the normal ranges of the individual as their reassigned gender.

For the whole group the mean Z score was 0.044 ±1.72 at the lumbar spine (L2-L4) and 0.42±1.11 at the femoral neck as expected in skeletally healthy subjects.

The grouped serial data showed an increase of density in the lumbar spine of 1.82%±2.6% per annum, and 0.38%±1.76% in the femoral neck. Mean time between DXA visits was 4.17 yr ± 1.76yr. These changes indicate that the age related bone loss seen in the "normal" range was not reflected over the relative short time in these subjects.

These finding are reassuring for the prescriber and recipient of hormone reassignment.

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BIGGER, FASTER, STRONGER - IMPROVEMENT IN EARLY OUTCOMES FOLLOWING USE OF ZOLEDRONIC ACID IN NEONATES WITH SEVERE FORMS OF OSTEOGENESIS IMPERFECTA

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Severe forms of Osteogenesis Imperfecta (OI) diagnosed in the neonatal period have been historically associated with chronic disabilities, including severe limitation of linear growth, multiple childhood fractures and impaired mobility. However, the early use of bisphosphonates in these patients leads to a dramatic improvement in functional outcomes compared to historical controls. We report the outcomes of 5 patients with OI who received their first infusion of Zoledronic Acid (ZA) 0.04mg/kg/dose, at less than 1 month of age.

Of 5 neonates, 4 had clinical evidence of fractures at birth (mean no. fractures 21, range 14 - 30), with the fifth presenting at day 21 with multiple fractures (n=8). Mean age at first ZA infusion in this group was 11 days (range 1 - 28 days). The treatment regime involved repeat ZA infusions at 4 monthly intervals, with a mean age at most recent follow up of 28.4 months (range 16 - 44 months). Median number of fractures occurring after the first infusion was 4 per infant (range 3 - 40). Three of our cohort are now walking independently, (mean age 19 months), a markedly improved outcome compared to historical, non-treated patients[1]. Of our remaining 2 children, one is crawling at 16 months. One infant, the most severe of the group, has had 40 fractures despite treatment but is sitting unassisted and rolling at 20 months. He has subsequently undergone bilateral femoral osteotomies to improve his prospects for weight bearing. While linear growth is still decreased compared to age matched controls (mean height SDS -2.71, range -0.25 to -5.85), this is again far greater than historical controls (mean Ht SDS -5.0[1]).

Video footage of a selection of these infants is offered in order to demonstrate their remarkable developmental progress, given the disease severity. We also report unexpected resistance to treating such severely affected infants across a range of medical and paramedical staff, given the poor previous outcomes experienced for other similarly affected infants.

(1) Munns CF,Rauch F,Travers R,Glorieux FH.Effects of iv pamidronate treatment in infants with osteogenesis imperfecta: clinical and histomorphometric outcome. J Bone Miner Res 2005;20(7):1235-43

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PREVALENCE OF HYPOVITAMINOSIS D IN JAPANESE PREGNANT WOMEN IN WINTER

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Serum 25-hydroxyvitamin D (25-OHD) concentrations are thought to accurately reflect vitamin D stores, and vitamin D deficiency causes secondary hyperparathyroidism, which can lead to osteomalacia, irreversible bone loss and increased risk of fracture. In addition, recent studies suggest that hypovitaminosis D in mother could influence neuromuscular diseases of their children in the future. We have recently reported that seasonal changes of 25-OHD concentrations in normal Japanese population (Ono et al., J Bone Miner Metab 23:147-151, 2005), and the existence of hypovitaminosis D especially in winter even in sunny area in Japan. The aim of the present study is to investigate the prevalence of hypovitaminosis D in Japanese pregnant women in winter. All of the participants were the residents of Tokai area in Japan (N35.3, E137.0). Serum concentration of 25-OHD in 20 pregnant women after 30th weeks of their gestation was determined by direct radioimmunoassay. Mean 25-OHD levels were 13.5ng/ml. Eighteen subjects out of 20 women showed hypovitaminosis D, which was defined as serum 25-OHD concentration was equal to or less than 20 ng/ml. Mean serum intact parathyroid hormone (iPTH), serum bone-specific alkaline phosphatase (BAP) and serum type I collagen N-terminal telopeptide (NTx) were 34.5pg/ml, 24.4U/l, 14.0nmolBCE/l, respectively. Serum 25-OHD levels were not associated with either iPTH, BAP, NTx, corrected calcium or phosphate concentrations in these subjects. There were four patients with hyperetention during pregnancy, and two mothers had babies with intrauterine growth retardation and one had her baby with major malformation. However, serum concentration of 25-OHD in these subjects were not lower than those in other subjects. In conclusion, our results suggest the high prevalence of hypovitaminosis D in Japanese pregnant women in winter without major effect on calcium-phosphate metabolism.

EFFECT OF PARATHYROID HORMONE DEFICIENCY AND EXCESS ON CORTICAL AND TRABECULAR MICRO-ARCHITECTURE

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Patients with secondary hypoparathyroidism (HypoP) have increased bone mineral density (BMD) while patients with primary hyperparathyroidism (PHTP) have reduced BMD at cortical sites but normal or high BMD at trabecular sites. These observations suggest that parathyroid hormone (PTH) excess produces cortical thinning by endocortical resorption but is anabolic at trabecular sites (1) . Recent work suggests that age-related intracortical remodelling produces coalescent cavities trabecularizing the cortex leading to cortical thinning from 'within' rather than by endocortical resorption (2) . We hypothesize that (i) trabecular number and thickness is reduced, not increased in PHTP. (ii) Cortical remnants outside the endocortical surface are 'seen' as trabeculae and factitiously elevate trabecular number and thickness. (iii) Cortical area, thickness and density is reduced because PTH increases intracortical porosity. (iv) HypoP patients have normal cortical area and thickness. We propose that endocortical resorption contributes minimally to cortical thinning while cortical density is increased due to reduced intracortical porosity. Trabecular thickness will be increased while numbers are reduced due to reduced age related perforation (which can increase trabecular numbers).

We assessed morphology using HR-pQCT in 5 patients with untreated PHPT, median age 58 years (range 30 - 73); 5 patients with surgically treated PHTP, median age 55 years (range 34 – 58) and 3 patients with post-surgical HypoP, median age 53 years (range 45 - 54). Morphology is expressed as z scores (number of standardised deviations from the sex- and age-specific mean). Results for treated and untreated PHPT were combined.

Tibial cortical area (mean z score 0.97 +/- SEM 0.72 vs -0.36 +/- SEM 0.22; p = 0.03) and cortical thickness (mean z score 0.91 +/- SEM 0.64 vs -0.29 +/- SEM 0.20; p = 0.03) was increased in HypoP but decreased in PHPT patients (refer to fig 1). Tibial trabecular parameters were normal in HypoP but were reduced in PHPT patients (refer to fig 1). Similar data were found in the radius (not shown).

While inferences are constrained by the small sample sizes in this pilot study, the data is suggestive that PTH excess is deleterious to both cortical and trabecular bone.

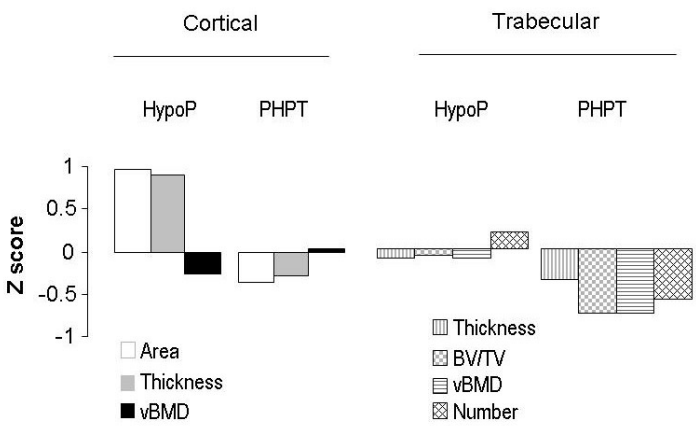

Fig 1. Tibial bone parameters

(1) Seeman E et al. Journal of Clinical Investigation 1982; 69(6): 1302 - 1309

(2) Zebaze R et al. Abstract 1101 ASBMR 2008

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POLYETHYLENE PARTICLES ACTIVATE PRO-OSTEOCLASTOGENIC SIGNALLING PATHWAYS IN HUMAN PRIMARY OSTEOBLASTS AND OSTEOCYTES

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The reaction of orthopaedic periprosthetic tissues to polyethylene (PE) wear particles is thought to account for much of the osteolysis associated with aseptic loosening and implant failure. While cells of the monocyte/macrophage lineage are implicated, evidence suggests that osteoblastic cells may also be affected by PE. In this study we developed a novel and robust *in vitro* cell culture system that replicates the 3-dimensional (3D) environment of the osteoblast, while juxtaposing the cells and PE particles. We report that normal human bone-derived cells (NHBC), shown previously to represent human primary osteoblasts, undergo differentiation in 3D culture into a mature osteocyte-like phenotype over a 21-28 day culture period, acquiring increased expression of osteocalcin (OCN), E11, DMP-1 and SOST/sclerostin mRNA, and developing an osteocyte-like morphology. While PE did not impair the ability of the cells to differentiate, NHBC responded to PE by increasing the expression of genes associated with support of osteoclast formation and activity (RANKL, IL-8 and M-CSF), and did so at a relatively late stage of their differentiation. Linear regression analysis of paired gene expression across the culture period indicated that PE may induce a switch in RUNX2 control of gene expression from matrix production (type I collagen) towards the expression of pro-osteoclastogenic genes. These results suggested that mature osteoblastic cells responded to PE directly. This was further evidenced by the induction by PE of the expression of RANKL mRNA in the mouse osteocyte cell line, MLO-Y4, and the absence of monocyte/macrophage contamination of the responding primary NHBC populations. In conclusion, our results demonstrate that mature osteoblasts and osteocytes respond directly to PE in a pro-osteoclastogenic manner, which may contribute to the osteolytic effect of PE in orthopaedic implant recipients.

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THE P.R176Q MUTATION IN FGF-23 GENE IS FIRSTLY FOUND IN A CHINESE FAMILY WITH AUTOSOMAL DOMINANT HYPOPHOSPHATEMIC RICKETS

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Objective: To investigate the FGF23 gene mutation in a Chinese family with autosomal dominant hypophosphatemic rickets (ADHR, MIM 193100).

Subjects and methods: 1. Subjects: This Chinese family consists of four family members. The proband is a 30 year old female. She presented progressive painful swelling of left ankle after a blunt trauma at 26 years of age. She was found to have severe hypophosphatemia. She had two times of fracture on both ankles. She did not have a history of rickets but had multiple tooth abscesses as a young adult. Her brother is 26 years old. He presented fatigue at 24 years of age but no hypophosphatemia. The parents of this family were more than 55 years old and they had no history of rickets or presence of hypophosphatemia. 2. After obtaining informed consent, blood samples were taken from the four family members and 50 ethnically-matched unrelated controls. Genomic DNA was isolated from extracted from peripheral blood by standard Phenol-chloroform extraction procedure. The FGF-23 gene fragments covering the entire coding region and intron/exon boundaries were amplified by PCR. The amplified products were directly purified and sequenced. Mutations were identified by comparing the sequences against the reference DNA of the FGF-23 gene.

Results: We detected a heterozygote missense mutation c.527G>A (p.R176Q) in the FGF-23 gene in three of the family members including the proband, her brother and their mother. The p. R176Q mutation was the cause of ADHR in this Chinese family.

Conclusions: We investigated the FGF-23 gene mutation in a Chinese family diagnosed with ADHR and detected c.527G>A (p.R176Q) mutation in three of the family members. We present the first report of p.R176Q mutation in FGF23 gene in a Chinese family with ADHR.

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THE RISK OF OSTEOPOROSIS RELATED FRACTURE IN DIABETIC PATIENTS

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We utilized placebo groups from pivotal risedronate trials to examine the risk of osteoporosis-related fractures in diabetic patients. Patients with diabetes, particularly those with type 2 diabetes, usually have a higher BMD than would be expected, but are nevertheless at increased risk of fracture.

About 5% (n=306) of patients from the VERT and HIP trials were identified as diabetic either via medical history or concomitant medication data. Patients were aged 79 years or younger. One hundred and seventy (55%) of the 306 patients were treated with oral hypoglycemic agents; none were on insulin.

Patients were recruited based on BMD in the osteoporotic range. On average, the diabetic patients had a greater BMI compared to non-diabetic patients 27.9 vs 25.4 ($p<0.0001$). The mean age of the diabetic patients (72y) was similar to the non-diabetic patients ($p=0.6011$). There was a statistically significant difference of 1 year in the average years since menopause, but this small difference was not clinically meaningful. Diabetic patients had statistically significantly ($p<0.001$) greater BMD values at baseline compared to non-diabetic patients (mean LS T-score -2.0 vs -2.6 and mean FN T-score -2.4 vs -2.6 ,respectively). BMD T-scores were significantly higher for diabetic compared to non-diabetic patients. Prevalent radiographic vertebral fractures for diabetic vs non-diabetic patients (61% vs 59% respectively) and osteoporosis-related non-vertebral fractures (8% vs 10%) were not statistically significantly different ,$p>.07$).

The incidence of vertebral fractures was similar between the diabetic and non-diabetic patients. However, diabetic patients had a statistical significantly greater risk of non-vertebral fractures (composite endpoint of the following sites: wrist, clavicle, humerus, pelvis, leg, and hip) relative to non-diabetic patients (RR=1.69, $p=0.019$).

At baseline, both diabetic and non-diabetic patients had the same prevalence of vertebral and non-vertebral fractures. BMD values were higher in the diabetic patients. Although vertebral fracture risk was the same in osteoporotic postmenopausal women with or without diabetes, the risk of non-vertebral fracture was higher in diabetic patients. Physicians need to be aware of the higher non-vertebral fracture risk in these patients.

Category 5. Bone Resorption and its Regulation

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OSTEOCYTE CELL DENSITY IS REDUCED DURING VITAMIN D DEPLETION AND PREDICTS OSTEOCLAST SURFACE AND BONE MINERAL VOLUME

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Although the association between hip fracture risk and vitamin D deficiency is well established, understanding of the cellular mechanisms is less clear. Previously, we have reported maintaining adequate levels of serum 25-hydroxyvitamin D (25D) prevent bone loss by reducing RANKL-mediated osteoclastic bone resorption in an animal model. Interestingly, it has been demonstrated that increased bone resorption is mediated by osteocytes (OCY) either via apoptosis or trans-cellular signalling. Hence, we have used our vitamin D-deplete animal model to investigate the relationship between trabecular bone OCY cell density and other bone variables. Briefly, vitamin D-depleted animals were fed one of several levels of vitamin D_3 (2IU-20IU/rat/d) with 0.4% dietary Ca from 3m of age. At 7m, the mean 25D level ranged from 20 nmol/L to 115 nmol/L which was demonstrated to be stable for at least 12 weeks. In addition to serum biochemical analyses, the distal femoral metaphysis from each animal was analysed on normal and decalcified 5 μm sagittal sections for standard histomorphometric variables, such as osteoclast surface (OcS, %), plus osteocyte numbers relative to bone mineral area (OCY/BMA, #/mm^2). As previously reported, trabecular bone volume (BV/TV) was positively related to circulating 25D ($R^2=0.51$, $P<0.001$), but not to serum $1,25(OH)_2D_3$, serum calcium nor parathyroid hormone (PTH) levels. The reduction in BV/TV as result of reduced serum 25D levels was negatively associated with increases in both RANKL:OPG mRNA ratio ($R^2=0.35$, $P<0.05$) and OcS ($R^2=0.36$, $P<0.05$). The OCY/BMA in trabecular bone was lowest in animals with 20nmol/L 25D ($P<0.01$) when compared to all other groups and positively correlated with serum 25D levels ($R^2=0.46$, $P<0.01$). Furthermore, of all the measures of bone formation and resorption, only OcS was negatively associated with OCY/BMA ($R^2=0.3$, $P<0.05$). OCY/BMA was also correlated

with BV/TV (R^2=0.23, P<0.05), suggesting that a low osteocyte cell density in trabecular bone may play a role in determining osteoclastic bone resorption and ultimately bone mineral volume. Measures of osteocyte survival/activity in this vitamin D-depletion animal model, will confirm whether osteoclast activity during vitamin D depletion is due partly to osteocyte signalling.

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NOVEL GERMACRANE SESQUITERPENE ESTERS INHIBIT NF-KB ACTIVITY AND RANKL-INDUCED OSTEOCLAST FORMATION AND BONE RESORPTION

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Osteoclasts are responsible for bone resorption and play a pivotal role in the pathogenesis of osteolytic disorders. NF-κB is a set of nuclear factors essential for osteoclast formation and survival. NF-κB signalling pathways are strictly regulated to maintain bone homeostasis by cytokines such as RANKL, TNF-α and IL-1. Abnormal activation of NF-κB in osteoclasts has been frequently observed in osteolytic bone diseases such as periprothetic osteolysis and arthritis, and this raises an intriguing possibility that NF-κB might serve as a therapeutic target for the treatment of osteolytic bone diseases. We have previously shown that sesquiterpene lactone Parthenolide attenuates osteoclast formation and function through the inhibition of NF-κB activity. Further screening of sesquiterpene structural analogues from a natural compound library identified 3 novel germacrane sesquiterpene bearing the same core carbon structure as parthenolide. These compounds were isolated from the whole plant of Salvia Roborowskii. Using primary bone marrow monocytes (BMMs) osteoclastogenic culture system, we examined the effects of these compounds on osteoclast formation. Bone resorption assay was employed to examine their inhibitory effects on the function of osteoclasts. Immunoblot analysis, together with luciferase reporter gene assay was used to dissect the molecular mechanism(s) underlying the observed effects of the compounds on RANKL-induced NF-κB and p-ERK signalling. We demonstrated that structural analogs of parthenolide dose-dependently inhibit RANKL-induced osteoclastogenesis in BMM cells as well as disrupting the ability of the osteoclasts to resorb bone. Compound A was identified to be the most potent amongst the 3 analogues. Luciferase reporter gene assay revealed that these compounds decrease RANKL-induced NF-κB activation. Furthermore, western blotting analysis also demonstrated that the compounds inhibit IκB-α degradation, but had little effect on RANKL-induced ERK phosphorylation. Taken together, these results indicate that structural analogues of parthenolide effectively inhibit osteoclast formation and function through the inhibition of NF-κB. We propose that novel germacrane sesquiterpene esters might provide therapeutic candidates for the treatment of osteolytic bone diseases associated with enhanced osteoclastic activity.

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ACUTE HYPOXIA AND OSTEOCLAST ACTIVITY: A BALANCE BETWEEN ENHANCED RESORPTION AND INCREASED APOPTOSIS

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Hypoxia is a central component of skeletal conditions including rheumatoid arthritis, pathological fracture, primary bone tumours and cancer metastases to bone. Hypoxia regulates gene expression via the transcription factor HIF (hypoxia-inducible factor), which is emerging as a central regulator of osteoblast function. Given the impact of osteoclasts, the primary mediators of pathological bone resorption, on the pathobiology of hypoxia-associated skeletal conditions, we investigated effects of hypoxia on human osteoclasts.

Mature osteoclasts were obtained by differentiation of PBMC with M-CSF + RANKL or by curettage of Giant Cell Tumour of Bone. Osteoclasts were exposed to a constant hypoxic environment then assessed for parameters including resorption (toluidine blue staining of dentine slices), membrane integrity (trypan blue exclusion), apoptosis (TUNEL, DAPI) and osteolytic enzyme activity (TRAP, Cathepsin K).

24 h exposure to 2% O_2 produced a 2.5-fold ($p<0.01$) increase in resorption associated with increased TRAP (40% increase, $p<0.01$) and Cathepsin K (15% increase, $p<0.01$) enzyme activity. HIF-1α protein was induced at 2% O_2 and HIF-1α siRNA completely ablated the hypoxic increase in osteoclast resorption ($p<0.001$) via a mechanism independent of VEGF. At the same time as increasing resorption, 24 h at 2% O_2 reduced the number of VNR-positive osteoclasts (76% reduction, $p<0.01$) and increased the proportion with compromised membrane integrity from 6% to

21% ($p<0.05$), with no change in total osteoclast number or the proportion of late stage apoptotic cells. Transient reoxygenation returned the percentage of trypan blue-positive cells to normoxic levels, suggesting that osteoclasts can recover from the early stages of cell death. Over 14-21 days, constant 2% O_2 dramatically inhibited osteoclast formation ($p<0.05$) and activity ($p<0.001$) , whereas hypoxia / reoxygenation enhanced osteoclast differentiation at this pO_2 ($p<0.001$).

We have described time- and O_2 concentration-dependent effects on the activity and viability of human osteoclasts. These results suggest that within the normal bone microenvironment of 7-9% O_2, osteoclast resorptive activity and viability is comparable with that under standard tissue culture conditions. However in diseased bone, where the pO_2 may fall to $\leq$2% O_2, a delicate balance between osteoclast activation and apoptosis, modulated by periods of transient reoxygenation, mediates pathological bone resorption.

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TIBIA BONE DENSITY LOSSES VIA PQCT IN WOMEN DURING PROLONGED BED-REST NOT PREVENTED BY NUTRITION COUNTERMEASURES OR COMBINED FLYWHEEL RESISTIVE EXERCISES AND SUPINE TREADMILL EXERCISE WITHIN LOWER BODY NEGATIVE PRESSURE

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Purpose: To examine the effect of prolonged bed-rest with countermeasures on tibia bone density in women.

Method: In the WISE2005 study, 24 healthy women underwent 60-days of 6° head-down tilt (HDT) bed-rest and were randomly assigned to either an inactive control group (CON), a nutrition (NUT; leucine-enriched protein diet) countermeasure group, or a group (EX) performing a mixed resistance ("flywheel" thigh and calf exercises; 2-3 days/week) and aerobic (supine treadmill within lower body negative pressure; 3-4 days/week) exercise countermeasure. We evaluated total, cortical and total bone density and mass of the distal tibia and cortical bone density and mass in the proximal tibia using peripheral quantitative computed tomography (pQCT-XCT2000, Stratec Medizintechnik, Germany) prior to bed-rest (BDC), on HDT days 15, 43, an on post-bed-rest recovery (R+) days 3, 90, 180 and 360.

Results: No influence of either of the countermeasures were seen on any of the distal and proximal tibia bone density and mass variables (group×study-date: F all<1.17, p all >.31). Strong statistical effects were seen however for changes in these variables (irrespective of subject-group) over the course of the study (study-date: F all≥12.84, p all<.0001). Post-bed-rest (R+3), total distal tibia bone density was 3.1(0.5)% lower than baseline in the CON group (p<.00001), 2.2(0.5)% lower in the EX group (p=.00001) and 3.2(0.5)% lower in the NUT group (p<.00001). One year post-bed-rest (R+360), total distal tibia bone density was marginally reduced in the EX and NUT groups (-0.9[0.6]% and -0.7[0.5]% respectively; p>.11) with a -1.8(0.5)% reduction (p=.00057) in the CON group, though this latter effect was largely due to one subject who showed no recovery.

Conclusion: This study is, to our knowledge, the first to examine distal tibia bone density losses during bed-rest in women. The loss of 3.1% of distal tibia total bone density in women is comparable to that seen in men after bed-rest. The limited effectiveness of either of the countermeasures in preventing bone atrophy suggests the exercises, as performed presented an insufficient stimulus for the retention of bone during bed-rest/spaceflight. Further work on countermeasure development is needed considering exercise type, duration, intensity and frequency.

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RANKL MUTANTS INHIBIT OSTEOCLASTOGENESIS AND BONE RESORPTION *IN VITRO*.

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Mutations within the TNF-like core domain of Receptor Activator of NF-κB Ligand (RANKL) have been recently reported in patients with osteoclast-poor autosomal recessive osteopetrosis (ARO). However, the functional consequence owing to RANKL mutations has not been well characterized. Here we describe the functional propensity of RANKL mutants in osteoclast differentiation, and their impact on RANKL-mediated signaling cascades.

Recombinant RANKL (rRANKL) mutants within the TNF-like core domain exhibited diminished osteoclastogenic potential as compared with wild-type rRANKL1 encoding the full TNF-like core domain (aa160-318). rRANKL mutants demonstrated reduced activation of NF- k B, IκB a degradation and ERK phosphorylation. In addition, we found that rRANKL mutants interfered with wild-type rRANKL-induced osteoclastogenesis with deletion mutant rRANKL5 (aa246-318) exhibiting the greatest inhibitory effect. The same mutant also significantly reduced wild-type rRANKL1 (aa160-318)-induced osteoclastic bone resorption *in vitro*. BIAcore assays demonstrated that rRANKL5 alone, lacking the AA" and CD loops, weakly bind to receptor RANK. Intriguingly, preincubation of mutant rRANKL5 with rRANKL1 prior to exposure to RANK enhanced the maximal binding level to RANK, indicating that rRANKL5 forms hybrid trimeric complexes with rRANKL1. Furthermore, RANKL mutants mimicking human RANKL V277 and M199K mutation in patients, impairs osteoclast differentiation and signaling. Taken together, these data lend support to the notion that the TNF-like core domain of RANKL contains structural determinants that are crucial for osteoclast differentiation and activation, thus providing a potential therapeutic regime for the treatment of osteoporosis and other osteolytic disorders.

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EPH-B4 FORWARD SIGNALLING IN OSTEOBLASTS IS TRIGGERED BY SOLUBLE CLUSTERED EPHRIN-B2 AND BLOCKED BY SPECIFIC PEPTIDE ANTAGONISTS.

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Ephrins and their receptors, Ephs, are a family of cell surface proteins that are involved in a number of contact-dependent control mechanisms in tissue development and maturity. It has been proposed that bidirectional signalling between Eph-B4 in osteoblasts and ephrin-B2 (efn-B2) in osteoclasts is involved in the coupling of bone resorption to bone formation. Ephrin binding causes clustering of Eph receptors, resulting in phosphorylation of tyrosine (Y) residues within the Eph cytoplasmic domain, a process known as forward signalling. Forward signalling through Eph-B4 increases osteoblast differentiation (1), and production of efn-B2 is enhanced by PTH/PTHrP within the osteoblast lineage (2). We have observed that soluble clustered efn-B2 can rapidly upregulate the phosphorylation of Y residues within the cytoplasmic domains of both Eph-B4 and Eph-B2 (an alternate receptor for efn-B2) on primary calvarial osteoblasts, Kusa 4b10 and UMR106.01 osteogenic sarcoma cells. The specificity of this effect was demonstrated by the use of a peptide antagonist, TNYLFSPNGPIARAW, which blocks the interaction between efn-B2 and Eph-B4, and inhibited the increase in Y phosphorylation of Eph-B4, but not Eph-B2. Conversely, another peptide antagonist, SNEWIQPRLPQH, which blocks the interaction between efn-B2 and Eph-B2, inhibited the efn-B2 induced increase in Y phosphorylation of Eph-B2, but not Eph-B4. Additionally, the recombinant extracellular domain of Eph-B4 (sEph-B4) also abrogated the phosphorylation effects mediated by clustered efn-B2. We also show that both TNYLFSPNGPIARAW and sEph-B4 inhibit mineralisation of osteoblastic Kusa 4b10 cells and the expression of mRNA for genes involved in osteoblast differentiation, including osteocalcin, bone sialoprotein, PTH1R, sclerostin, dentin matrix protein-1 and Bril. These results demonstrate that forward signalling through Eph-B4 can be specifically triggered by efn-B2 and blocked by specific peptide antagonists. The effects and specificity of clustered Eph-B4 onto efn-B2 Y phosphorylation (reverse signalling) are currently under investigation. By understanding the interactions between ephrin ligands and Eph receptors and their immediate and downstream effects, we can better understand how osteoblasts and osteoclasts communicate to regulate bone remodelling.

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MEF2 AND MITF COOPERATE WITH NFATC1 TO ACTIVATE V-ATPASE SUBUNIT D2 PROMOTER IN RANKL-INDUCED OSTEOCLASTOGENESIS

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The V-ATPase d2 protein is an important V-ATPase subunit that regulates bone homeostasis, however data regrading the regulation of d2 promoter is lacking. In this study, we investigate the regulation of the V-ATPase d2 expression during RANKL-induced osteoclastogenesis. Our data revealed that V-ATPase d2 gene expression is highly up-regulated by RANKL as compared to other pro-osteoclastogenic stimulatory factors, including M-CSF, TNF-α and LPS. An NFATc1 binding site was mapped to a region within the V-ATPase d2 promoter of -555 to -561 bps from the translation start site of d2. Intriguingly, the activation of d2 promoter by NFATc1 was co-activated by microphthalmia-associated transcription factor (MITF) and myocyte enhancer factor 2 (MEF2), respectively. In contrast, MITF and MEF2 did not coactivate d2 promoter. Using a combination of ChIP assay, EMSA and luciferase reporter studies, we further demonstrate the presence of NFATc1, MITF and MEF2 binding sites within the d2 promoter. Moreover, targeted mutation of these NFATc1, MITF and MEF2 binding sites was found to diminish the activation of d2 promoter. Immunostaining showed that MEF2 and NFATc1 proteins were co-localized in the nucleus of mature osteoclasts and MITF was also translocated from the cytoplasm to the nucleus following RANKL treatment. Moreover, when compared with GFP control group, over-expression of either MITF or a constitutively active form of MEF2-VP16 in RAW264.7 cells not only increased osteoclastogenesis, but also enhanced d2 gene expression with or without RANKL stimulation. Taken together, our data provide evidence that MEF2 and MITF collaborate with NFATc1 to activate V-ATPase d2 promoter in RANKL-induced osteoclastogenesis

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INHIBITION OF BOTH CLASS I AND CLASS II HISTONE DEACETYLASES IS REQUIRED TO EFFECTIVELY INHIBIT OSTEOCLAST BONE RESORPTION IN VIVO AND IN VITRO.

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Histone deacetylase inhibitors (HDACi) are a novel class of drugs reported to inhibit cancer cell growth, inflammation and osteoclast bone resorption. The aim of this study was to determine if inhibiting one class alone or inhibiting class I and II HDACs simultaneously is more effective at inhibiting osteoclasts. The novel dual class I and II HDACi 1179.4b, class I HDACi, MS-275, and class II HDACi, 2664.12, were investigated. The drugs were assessed using an in vitro model of human osteoclast formation and an in vivo mouse model of periodontal disease. In the in vitro assay osteoclasts were generated from human peripheral blood mononuclear cells by stimulation with RANKL and M-CSF over 17 days culture. The expression of tartrate resistant acid phosphatase (TRAP) and the ability to resorb dentine were assessed to determine osteoclast formation and activity. The HDACi were tested at concentrations between 0.2 nM and 100 nM. 1179.4b and MS-275 were also investigated in a mouse model of periodontitis in which disease was induced by an oral inoculation with P.gingivalis bacteria over a period of 81 days. The effect of the HDACi on alveolar bone loss was determined using a recently developed method of live animal micro computed tomography. The dual inhibitor, 1179.4b, inhibited osteoclast activity in vitro significantly reducing the number of TRAP positive cells and resorption (IC50 100nM). However, the combination of MS-275 and 2664.12 markedly inhibited osteoclast activity at concentrations similar to the dual inhibitor (IC50=0.35nM). In the mouse model of periodontal disease 1179.4b was found to significantly reduce the amount of alveolar bone resorption ($p=0.011$). MS-275, however, did not inhibit alveolar bone resorption. This data shows that inhibition of both class I and II HDACs simultaneously is required to effectively inhibit osteoclast activity in vivo and in vitro. In addition, dual class HDACis, such as 1179.4b, may be useful for the treatment of bone loss diseases.

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OSTEOCLAST INHIBITORY LECTIN (OCIL), AN IMMUNE CELL PRODUCT THAT IS REQUIRED FOR NORMAL BONE PHYSIOLOGY *IN VIVO*

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NK cell C-type lectins have a described role in autoimmune cell function and we have previously reported their emerging role to regulate osteoblast formation from mesenchymal-derived stromal cells and osteoclast formation or activity. We now report the first demonstration for a significant role for a NK cell C-type lectin in bone metabolism *in vivo*. Mice lacking the NK cell C-type lectin OCIL (or clrb) appeared healthy and were fertile, and phenotypic abnormalities were limited to bone. Loss of OCIL resulted in osteopenia in adult mice as a result of increased osteoclastogenesis and decreased bone formation. Enhanced osteoclast formation was apparent when either bone marrow or splenic cultures from *ocil* $^{-/-}$ mice were cultured *in vitro* relative to wild type (WT) cultures; increased osteoclast numbers were also detected transiently *in vivo*. The enhanced osteoclastic activity led to elevated serum calcium levels in 16-week old *ocil* $^{-/-}$ mice and suppression of circulating PTH in the 10-week old *ocil* $^{-/-}$ mice compared to WT control mice. Using histomorphometric analysis, what was apparent in 10-week and 16-week old *ocil* $^{-/-}$ mice was a significant reduction in trabecular bone volume and trabecular number together with reduced levels of bone formation rate (BFR) (both in trabecular and cortical bone) in both male and female *ocil* $^{-/-}$ mice at 10 and 16 weeks of age. Static markers of bone formation *in vivo* (such as osteoblast number/surface, osteoid volume/ surface/ thickness) showed no significant changes in male or female *ocil* $^{-/-}$ mice. Consistent with the low trabecular BFR and reduced periosteal mineral apposition rate in adult *ocil* $^{-/-}$ mice *in vivo*, assessment of mRNA levels of various osteoblast-specific marker genes in undifferentiated primary calvarial *ocil* $^{-/-}$ cells *in vitro* (*ex vivo*), revealed a significantly altered phenotypic profile of these cells with respect to calvarial cells from WT mice. Osteoblast phenotypic markers such as Col1-A1, ALP, BSP, and osteocalcin, including the transcription factor osterix, and Bril , were all significantly reduced in *ocil* $^{-/-}$ calvarial cells compared to WT controls. Collectively our data suggest that OCIL is a physiological negative regulator of bone turnover, and may further the links between immune-cell function and bone physiology.

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THE REGULATION OF PROTEIN KINASE C Δ IN BONE HOMEOSTASIS

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Bone homeostasis is maintained by the bone remodelling process which involves bone resorption by osteoclasts and bone formation by osteoblasts. Disruption of this balanced process is associated with bone diseases. Osteoclasts are formed from the fusion of macrophage precursor cells stimulated with the receptor activator of NF-κB ligand (RANKL). The signalling pathways that regulate osteoclast formation are not fully understood. Protein kinase C (PKC) has been implicated in regulating RANKL signalling pathways and osteoclastogenesis. To further investigate specific the role of PKC isoforms in osteoclast biology, we have compared the gene expression profile of PKC isoforms in RAW cell and primary bone marrow monocyte (BMM) derived osteoclasts and found that PKCδ is highly expressed in osteoclasts. Further studies using isoform-specific agonists and antagonists of PKC activity support a role for PKCδ in osteoclasts. Inhibition of PKCδ by Rottlerin inhibited osteoclastogenesis and bone resorption, whereas activation of PKCδ by Bryostatin 1, enhanced osteoclastogenesis and osteoclast size. RT-PCR showed that the expression of osteoclast fusion gene DC-STAMP is up-regulated in Bryostatin 1 treated cells. Using luciferase reporter gene assays, we showed that the expression of constitutively active and dominant negative PKCδ mutants regulate NFATc1 and NF-κB transcriptional activity, which are essential for osteoclastogenesis. Interestingly, mCT and histology analysis demonstrates that PKCδ deficient (PKCδ-/-) mice exhibit an osteopetrotic (increased bone mass) phenotype. Alcian blue staining showed the presence of cartilaginous bars in the trabecular bone of PKCδ-/- mice consistent with an

osteopetrotic phenotype. These findings suggest PKCδ mediates bone homeostasis via the regulation of osteoclast differentiation.

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CORTICAL THICKNESS CHANGES IN RAT TIBIAE: THE EFFECT OF OVX AND BISPHOSPHONATE TREATMENT

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The goal of anti-osteoporosis treatments – such as bisphosphonates which suppress osteoclastic function – is to improve bone strength, and therefore decrease fracture risk. Along with bone mass and microarchitecture, bone dimensions such as the external diameter and cortical thickness play major roles in determining bone strength. The objective of this study was to quantify the effect of oviarectomy and also bisphosphonate treatment on the cortical thickness of rat tibiae.

Thirty two-month-old Sprague-Dawley rats were randomly assigned into three equal groups: oviariectomised (Ovx), oviariectomised plus Zoledronic acid treatment (Tx) and sham operated (Control), where the ovaries were exposed but not removed. The right tibia of each rat was scanned at baseline, and at weeks two, four, eight and 12 using a Skyscan-1076 micro-CT system. Corresponding volumes were selected in each dataset, and the cortical and trabecular bone was separated using a modified dual threshold segmentation method described by Buie et al. [1]. The true cortical thickness at each point in the volume was measured using the segmented 3D data.

The mean baseline cortical thickness was the same for the three groups (300 um). Over the 12 weeks mean cortical thickness increased significantly for all groups, with the Tx group showing the largest increase (620 um), and the Ovx group the smallest (470 um). The greatest changes occurred early in the study, and the thickness did not increase uniformly around the cortex.

Typically only a mean cortical thickness is reported in the literature [2], and the actual changes in cortical thickness that occur over time and with different treatments are not well understood. The results from this study help to understand the how cortical thickness changes in response to oviarectomy and bisphosphonate treatment.

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SKELETAL ACTIONS OF FIBROBLAST GROWTH FACTOR-8 *IN VITRO*

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The fibroblast growth factors (FGFs) are a group of at least 25 structurally related peptides that are involved in many biological processes. Some FGFs are active in bone, including FGF-1, FGF-2, FGF-18 and recent evidence indicates that FGF-8 is osteogenic, particularly in mesenchymal stem cells. In the current study, we found that FGF-8 was expressed in rat primary osteoblasts, and in osteoblastic UMR-106 and MC3T3-E1 cells. This molecule potently stimulated the proliferation of these osteoblastic cells in a dose-dependent manner, while inhibiting the formation of mineralized bone nodules in long term cultures of osteoblasts. The proliferation assays showed that FGF-8b was more potent than FGF-8a. FGF-8 induced the phosphorylation of p42/p44 mitogen-activated protein kinase (MAPK) in osteoblastic cells, however its mitogenic actions were not blocked by the MAPK kinase (MEK) inhibitor, U0126. Interestingly, FGF-8, unlike other members of the family, inhibited osteoclastogenesis in mouse bone marrow cultures predetermined to produce pre-osteoclasts, despite increasing the receptor activator of NFκB Ligand (RANKL)/OPG expression ratio. However, FGF-8 did not affect osteoclastogenesis in $RAW_{264.7}$ cells (a macrophage cell line, devoid

of stromal-cells) exogenously stimulated by RANKL. FGF-8 showed no effect on mature osteoclast function, as assessed in rat calvarial organ cultures and in a pit formation assay using isolated mature osteoclasts.

In summary, we have demonstrated that FGF-8 is active in bone cells, stimulating osteoblast proliferation in a MAPK-independent pathway and inhibiting osteoclastogenesis via a RANKL/OPG-independent mechanism. These data suggest that FGF-8 may have a physiological role in bone acting in an autocrine/paracrine manner.

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EFFECT OF LYSOPHOSPHATIDIC ACID (LPA) ON OSTEOCLASTOGENESIS.

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Lysophosphatidic acid (LPA) is a bioactive lipid with a growth factor-like activity on a large range of cell types. Among bone cells, LPA was shown to act on osteoblasts to induce cell proliferation, migration, differentiation and blebbing, and on osteocytes to mediate dendrite outgrowth. However, the role of LPA on osteoclasts is totally unknown. We have shown that, in an experimental osteolytic bone metastasis model where osteoclasts are massively recruited and activated, systemic treatments of mice with a blocker of LPA signaling inhibited osteolytic lesions while the progression of bone metastases. Only partial effect was mediated on cancer cells suggesting a dual effect on resorbing cells. Therefore, to address the role of LPA on osteoclast functions, we first check for the presence of receptors specific for LPA on osteoclasts. We found that, *in vitro*, mature osteoclasts derived from mononucleated cells isolated from bone marrow (BMMC) and spleen, treated with M-CSF and RANK-L, expressed mRNAs for LPA receptors (LPA_1, LPA_2, LPA_3). Cotreatments of BMMC with M-CSF, RANK-L and Ki16425 or VPC12249, which are both antagonists of LPA_1 and LPA_3 receptors, inhibited osteoclastogenesis (Ki16425>VPC12249). Moreover, we observed that continuous incubation of BMMC with Ki16425 affect the formation of multinucleated osteoclasts. Indeed, Ki16425 induced a 30% inhibition of osteoclasts with a small number of nuclei (3 to 5) as opposed to a 95% inhibition of osteoclasts with a high number of nuclei (6 to 10, and more than 10). This suggested that LPA might be essential for osteoclastogenesis temporally during the fusion stage of osteoclast precursors. We address this assumption by performing sequential treatments of BMMC with Ki16425. We observed that, even if Ki16425 inhibited the formation of high multinucleated osteoclasts (6-10 and >10 nuclei) when administered only during the proliferation stage (day 1 to 3, inh. 30%) or the differentiation stage (day 5 to 8, inh. 50%), Ki16425 treatment induced a higher inhibition when delivered only during the fusion stage (day 3 to 5, inh. 70%). Altogether, these results indicate that by acting through its receptors, LPA is essential for osteoclastogenesis *in vitro* and suggest that interfering with LPA signaling might be useful for patients with pathological increases of osteoclast formation.

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NEW INSIGHTS INTO THERAPEUTIC DRUG INTERVENTIONS FOR CATABOLIC BONE DISEASES USING AN IN-SILICO MODELING APPROACH

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The purpose of this study is to investigate possible therapeutic drug interventions applied to catabolic bone diseases. Among the many catabolic bone diseases we restrict our focus to those related to the RANK-RANKL-OPG system. In order to test various therapeutic strategies we employ a previously proposed bone cell dynamics model which uses cell numbers and bone volume as output functions. Clinically, the latter two quantities can be linked to the action of drugs on bone volume (or bone mass) and bone turnover. The bone model contains the RANK-RANKL-OPG signaling system between osteoblasts and osteoclasts together with the action of TGF-be on bone cells. We use the entire parameter space of the model as possible therapeutic interventions - these include differentiation and apoptosis rates of osteoblasts and osteoclasts together with components of the RANK-RANKL-OPG system (such as for example RANKL production). Based on the numerical simulations we can identify three individual patterns of bone turnover for every successful therapy, i.e., those which are able to store bone volume back to normal. The first “pro-anabolic” pattern is characterized by high bone turnover and is associated with changes of osteoblast differentiation and apoptosis

rate. The second "anti-catabolic" pattern is characterized by rather normal bone turnover caused by changes of differentiation and apoptosis rates of osteoclasts together with parameters of the RANK-RANKL-OPG system. Finally, the third pattern shows mixed properties, i..e, have normal active osteoblast and osteoclast cell numbers while having rather low osteoblast precursor numbers associated with changes in the differentiation rate of osteoblast precursor cells.

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MICE LACKING AMP-ACTIVATED KINASE (AMPK) SUBUNITS B1 OR B2 HAVE LOW BONE MASS, WHILE AICAR ACTS AMPK-INDEPENDENTLY TO INCREASE OSTEOCLAST FORMATION.

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AMPK is a ubiquitously expressed energy sensing enzyme that regulates whole body and cellular energy homeostasis and protects cells from stresses that deplete the energy charge (i.e. increase in the AMP:ATP ratio), such as exercise, hypoxia and heat shock. However the role of AMPK in the bone has not been investigated.

AMPK is a heterotrimeric α, β, γ enzyme with two β-subunit isoforms. We developed a β1 knockout ($\beta1^{-/-}$) and a β2 knockout ($\beta2^{-/-}$) mouse line. Both types of knockout mice displayed reduced functional AMPK in many cell types, and displayed abnormally low trabecular volume and trabecular number compared to matched wild type littermate controls. $\beta1^{-/-}$ x $\beta2^{-/-}$ crosses yielded no $\beta1^{-/-}$/ $\beta2^{-/-}$ offspring (which would lack AMPK), indicating embryonic lethality.

Since these observations suggested that AMPK significantly influence bone metabolism, we investigated effects of a direct AMPK activating compound, AICAR. This compound is converted in cells to the AMP analogue ZMP (through the intracellular action of adenosine kinase) which mimics the effect of cellular metabolic stress. AICAR administration caused 10 week old male mice to rapidly lose trabecular and cortical bone mass (determined by pQCT and histomorphometry) with greatly elevated osteoclast numbers in an adenosine-kinase dependent manner. AICAR also stimulated osteoclast formation (but not resorptive activity) in several models of *in vitro* osteoclast differentiation, suggesting a direct pro-osteolytic effect of this compound on osteoclast progenitors. However, β2 AMPK subunit was not detected in macrophage/osteoclast lineage cells in wild type or $\beta1^{-/-}$ mice, and indeed we determined that in the latter, macrophage lineage cells were AMPK null. Consistent with our evidence that AICAR acts in an AMPK independent manner to increase osteoclast formation, AICAR greatly stimulated osteoclast formation from $\beta1^{-/-}$ mouse bone marrow.

Our data suggest that while AMPK significantly influences bone mass, it plays no cell-autonomous role in physiological osteoclast formation or activity. This study also showed that AICAR, a compound proposed as a useful treatment for insulin resistance, has powerful pro-osteolytic actions through effects on macrophage/osteoclast lineage cells which are, nonetheless, independent of AMPK.

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OSTEOMACS: OSTEOCLAST PRECURSORS DURING INFLAMMATORY BONE DISEASE BUT REGULATORS OF PHYSIOLOGIC BONE REMODELING

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We have identified a population of resident tissue macrophages (OsteoMacs), defined by expression of F4/80 and anatomical location that are present within osteal tissues. OsteoMacs regulate in vitro mineralization by osteoblasts and are required for the maintenance of mature osteoblasts in vivo. The close lineage relationship of macrophages and osteoclasts lead us to investigate whether OsteoMacs function as osteoclast precursors. Primary OsteoMacs isolated from endosteal bone surfaces formed TRAP+ osteoclasts in the presence of RANKL (40ng/ml) and CSF-1, confirming

that like many myeloid cell populations, they can be driven to form osteoclasts in vitro. Interestingly, similar treatment of calvarial digest cultures, that contain both osteoblasts and OsteoMacs, produced two distinct populations: F4/80+/TRAP- OsteoMacs and mononuclear/binuclear F4/80-/TRAP+ cells. Therefore, in this more physiologic mixed culture, OsteoMacs are not osteoclast precursors. Immunohistochemical analysis of the metaphyseal region in bone sections confirmed that F4/80+ OsteoMacs do not express TRAP and mononuclear TRAP+ cells do not express F4/80, indicating that during physiologic bone turnover OsteoMacs are not osteoclast precursors. Comparison of Mac-3 and F4/80 distribution within the metaphyseal zone identified that a Mac-3+/F4/80- myeloid population predominates in the primary spongiosa, a region rich in osteoclasts, confirming that other potential osteoclast precursor populations are present in the appropriate anatomical location. The bone microenvironment during inflammatory bone disease is greatly altered and in response to these conditions the in vivo osteoclast precursor population may change. In adjuvant-induced arthritis mononuclear transition cells expressing both F4/80 and TRAP were associated with sites of resorption indicating that in this pathological setting macropahges contribute to the osteoclast precursor pool. Finally, we identified a canopy structure encapsulating mouse bone remodelling units, similar to that reported in human bone, and demonstrated that the canopy cell is an F4/80+ OsteoMac. These data suggest OsteoMacs, like other tissue macrophages, are highly adaptive and their roles in bone biology change depending on the local microenvironment. Specifically during pathological conditions OsteoMacs may function as osteoclast precursors. However, during physiologic conditions they may be important coordinators of bone remodelling through regulation of osteoclast recruitment, formation and activity as well as osteoblast function.

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LATE EXPRESSION OF C-FOS DURING OSTEOCLAST DIFFERENTIATION DETERMINES OSTEOCLAST SURVIVAL AND BONE MASS

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The transcription factor c-Fos is essential for osteoclast differentiation. c-Fos-deficient mice lack osteoclasts and show severe osteopetrotic phenotype due to impaired expression of the c-Fos target gene *Nfatc1*. c-Fos expression in osteoclastgenesis is biphasic: early expression is observed within 30 min and late expression 48 h after RANKL treatment. However, distinct roles of early and late expression of c-Fos are unknown. In this study, we examined the role of c-Fos late expression in osteoclastgenesis. First, we prepared bone marrow-derived macrophages from *Fos*flox/- mice, and treated these cells with RANKL. Twenty-four hours after treatment, we infected cells with adenovirus expressing Cre-recombinase to remove the late expression of c-Fos. As a result, expression of *Nfatc1*, *Acp5* and *Mmp9* was reduced, the number of multinuclear TRAP-positive cells was decreased, and resorption activity was diminished. We then removed c-Fos late expression *in vivo* by generating *Fos*flox/- mice carrying the *Cathepsin K*-Cre recombinase knock-in locus. During osteoclastgenesis using bone marrow-derived macrophages prepared from these mice, we confirmed that late expression of c-Fos was removed while early expression of c-Fos was intact. Removal of c-Fos late expression by *Cathepsin K*-driven Cre-recombinase reduced expression of *Nfatc1*, number of multinuclear TRAP-positive cells, and resorption activity during RANKL-induced osteoclastgenesis. Unexpectedly, however, removal of c-Fos late expression prolonged survival of osteoclasts *in vitro*. Analyzing tibiae of these mice using micro-computed tomography and histomorphoretry revealed that the mouse lacking late expression of c-Fos decreased bone mass. These findings suggest that late expression of c-Fos during osteoclast differentiation enhances full maturation and limits survival of osteoclasts.

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THE COMBINATION OF CALCIUM WITH VITAMIN D IS A MORE EFFECTIVE SUPPRESSOR OF PARATHYROID HORMONE THAN EITHER GIVEN ALONE

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Introduction : Calcium with vitamin D is more effective in fracture prevention than either D alone. We postulated that the combination was a more effective suppressor of parathyroid hormone (PTH) than either alone and test this hypothesis in 20 volunteer women aged 59-75 with initial serum 25OHD below 60 nmol/L.

Methodology : Because vitamin D takes weeks to exert its actions whereas the effect of calcium on PTH immediate, we randomly allocated eleven to one week of calcium 1,000 mg daily followed by 7 weeks of Ca and D_3 1,000 i.u. daily and nine to 7 weeks of D_3 followed by one week of D_3 and Ca. Fasting blood samples were obtained at baseline, after one week of Ca or 7 weeks of D_3 and at the end of 8 weeks. We measured Total Ca, Phosphate, PTH and CTX in addition to albumin, globulins, anion gap and bicarbonate. Ionised calcium was calculated from total calcium and the four ligands.

Results : The two subgroups did not differ significantly from each other at baseline in any measured variable. Basal values and effects of treatments are shown in the Table. Calcium (whether given first or second) significantly raised Ca, Ca^{++} and P and lowered PTH and CTX. D_3 (first or second) did qualitatively the same but only the suppression of PTH and CTX were significant. Both treatments had greater and generally more significant effect than either alone. 25OHD rose from 48.1 nmol/L (SD 9.8) to 85.0 (SD 23.0) after 7 or weeks.

Initial values of measured variables, change on three regimens and final values in 20 postmenopausal women

		Change			
Variable	Basal	After Ca	After D_3	After both	Final value
		Change			
Total Ca (mmol/L)	2.36	0.055 **	0.010	0.070 ***	2.43 ***
Ionised Ca (mmol/L)	1.22	0.020 *	0.018	0.028 **	1.25 **
Phosphate (mmol/L)	1.11	0.019 **	0.027	0.060 *	1.69 *
PTH (pmol/L)	6.12	-0.65 *	-0.75 *	-1.53 **	4.60 **
CTX (ng/L)	439	-114 ***	-39 *	-161 ***	278 ***

*** P<0.001 ** P<0.01 * P<0.05 for significance from baseline

Conclusions: The combination of calcium and vitamin D was a more effective and significant suppressor of PTH than either alone.

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THE CALCITONIN RECEPTOR ON OSTEOCLASTS PLAYS A PHYSIOLOGICAL ROLE TO PROTECT AGAINST INDUCED HYPERCALCAEMIA IN MICE

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We have previously demonstrated that the calcitonin receptor (CTR) plays a biological role to protect against induced hypercalcaemia in mice (1). The aim of the present study was to investigate the mechanism by which the CTR exerts this effect using mice in which we have deleted the CTR either globally (global-CTR KOs) or specifically within osteoclasts (OCL-CTR KOs). At 6 weeks of age, mice were fed a low calcium diet for 2 weeks after which hypercalcaemia was induced by treatment with 0.5ug 1,25-dihydroxyvitamin D_3 (1,25D) on 2 consecutive mornings.

Total serum calcium (Ca) levels were measured immediately prior to, and 50.5 hours post-first 1,25D injection. Mice were then sacrificed and femora removed for histomorphometric analysis, and RNA was extracted from tibias. No differences were observed in baseline serum Ca and PTH levels between control and CTR KO genotypes, consistent with our hypothesis that the CTR on osteoclasts plays a modest physiological role in regulating bone homeostasis in the basal state. However, peak serum total Ca levels at 50.5 hours following 1,25-dihydroxyvitamin D_3 induced hypercalcaemia were greater in global-CTR KOs versus controls by 35% (1.2mM) ($P<0.05$) in males and by 31% (1.0mM) ($P<0.05$) in females. Similarly, serum total Ca levels in OCL-CTR KOs were greater than controls by 24% (0.7mM) ($P<0.05$) in males and by 19% (0.6mM) ($P<0.05$) in females. The observed increase in serum Ca in the CTR KOs was attributed at least in part to increased bone resorption compared to controls. Osteoclast surface area/ mineral surface was increased in global-CTR KOs compared to controls by 127% ($P<0.05$) in males and increased by 304% ($P<0.05$) in females. This was accompanied by an increase in the ratio of receptor activator of nuclear factor-kB (RANKL): osteoprotegerin mRNA in the tibias of male global CTR-KOs versus controls. Taken together our data suggest that the biological role of the CTR to protect against induced hypercalcemia in mice is primarily mediated via its inhibitory action on osteoclasts.

(1) Davey RA, Turner A et al. The Calcitonin Receptor Plays a Physiological Role to Protect Against Hypercalcemia in Mice. JBMR (2008) 23:1182-93

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ANALYSIS OF PROSTAGLANDIN RECEPTOR SUBTYPE EXPRESSION AND PRIMARY PROSTANOID RELEASE FROM TNF_A AND LPS – STIMULATED HUMAN MACROPHAGES AND OSTEOCLASTS

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Prostaglandins (PGs) have long been implicated as important mediators of inflammation in rheumatoid arthritis, and inhibition of PG biosynthesis with COX inhibitors provides effective therapy. A complete knowledge of prostanoid receptor pharmacology offers an opportunity for more targeted therapy. To this end, the present studies were intended to provide detailed analysis of prostanoid receptor expression by RT-PCR in human peripheral blood monocytes (PBMCs), macrophages, and osteoclasts. Macrophages were obtained by M-CSF treatment of PBMCs and osteoclasts were derived from macrophages by RANKL treatment. In addition, the release of the primary prostanoids PGD_2, PGE_2, $PGF_{2\,a}$, PGI_2 and thromboxane A_2 (TxA_2) from stimulated macrophages was quantified by LC-MS.

No EP_1 or EP_3 prostanoid receptor expression was apparent in PBMCs, macrophages, or osteoclasts under any condition. EP_2, EP_4, FP, and IP mRNA expression was apparent in all cell types studied and was not upregulated by TNF $_a$ or LPS: the relative abundance was EP_2 > IP> EP_4 > FP. Differences in expression were observed for other receptors. TP receptors were not expressed in PBMCs but TP transcripts were found in macrophages and osteoclasts. Conversely, DP_2 (CRTH2) receptors were expressed in PMBCs but not in osteoclasts or macrophages. DP_1 receptors were down-regulated when monocytes differentiated to macrophages and osteoclasts, and up-regulated in macrophages with LPS and TNFα stimulations.

LPS produced a substantial increase in prostanoid release from macrophages. TxA_2 release, measured as TxB_2, was the most prominent prostanoid released at about 8X PGE_2 and $PGF_{2\,a}$. PGD_2 was less abundant and no PGI_2 release (measured as 6-Keto $PGF_{2\,a}$) was detected. Since EP_1, EP_3, and DP_2 receptor expression was absent in macrophages and osteoclasts it seems reasonable to conclude that these three receptors cannot play a functional role in these human cells.

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GPR55: A NOVEL CANNABINOID RECEPTOR INVOLVED IN THE REGULATION OF OSTEOCLAST FUNCTION AND BONE MASS

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GPR55 is a poorly-characterised G protein-coupled receptor that is activated by endogenous, synthetic and plant cannabinoids as well as the endogenous lipid lysophosphatidylinositol (LPI). Given that the classical cannabinoid receptors CB_1 and CB_2 appear to play a role in bone physiology, we examined the role of GPR55 in bone cells.

Using immunostaining and quantitative PCR we found that GPR55 is expressed in human and mouse osteoclasts and osteoblasts. To study the functional effect of GPR55, cultures of osteoclasts (generated from human monocytes or mouse bone marrow macrophages) and mouse calvarial osteoblasts were treated with O-1602, a synthetic GPR55 ligand. O-1602 had little effect on osteoblasts but did increase the proportion of polarised osteoclasts (with an F-actin ring) and increased resorption pit area *in vitro*. For example at 50nM, the number of human osteoclasts with F-actin rings was 236 % ± 31 of control cultures ($P<0.01$) and resorption area was 217 % ± 28 of control cultures ($P<0.01$). Furthermore, the GPR55 antagonist cannabidiol (CBD, 500nM) significantly prevented the increase in osteoclast polarisation and bone resorption that occurred with 50nM O1602 treatment ($P<0.0001$, $n = 4$). Consistent with an increase in cytoskeletal polarisation, short term treatment of osteoclasts with 1µM O-1602 or LPI caused activation of Rho and ERK1/2. These activating effects of O-1602 and LPI on osteoclasts were attenuated either by the GPR55 antagonist cannabidiol or in $GPR55^{-/-}$ cells. This demonstrates for the first time that GRP55 agonists affect the activity of osteoclasts *in vitro.*

Consistent with a role for GPR55 in stimulating osteoclast function, histomorphometric and µCT analysis of the long bones from male $GPR55^{-/-}$ mice revealed a significant increase in the volume and thickness of trabecular bone in the proximal tibia and distal femur, as well as increased numbers of morphologically-inactive osteoclasts and the presence of unresorbed cartilage in the trabecular bone. Osteoblast parameters were not altered in $GPR55^{-/-}$ mice. Taken together, these data suggest that, in addition to CB_1 and CB_2, GPR55 plays a role in bone physiology by regulating osteoclast number and function. Furthermore, endocannabinoids that were previously considered to act via CB_1/CB_2 may act via GPR55.

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PREVENTION OF NECROTIC ACTIONS OF NITROGEN-CONTAINING BISPHOSPHONATES (NBPS) IN MICE BY NON-NBPS (CLODRONATE AND ETIDRONATE)

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Introduction: Nitrogen-containing bisphosphonates (NBPs) have powerful anti-bone-resorptive effects (ABREs). However, they have inflammatory side effects (such as fever, increase in acute-phase proteins, gastrointestinal disturbance, and ophthalmic inflammation). Recent clinical applications have disclosed an additional, unexpected side effect, osteonecrosis of jaw bones (ONJ). We previously found in mice that clodronate and etidronate (non-NBPs), when co-administered with alendronate (an NBP), inhibited the latter•fs inflammatory effects1-3. However, etidronate also reduced the ABRE of alendronate3. Zoledronate is the strongest anti-bone-resorptive NBP, but it is also associated with the highest incidence of ONJ. The mechanism underlying NBP-associated ONJ is unclear. Methods & Results: Here, we found the following. (a) Subcutaneous injection of NBPs into ear-pinnas induced inflammation and then necrosis of the ear skin (relative potencies: zoledronate >> pamidronate ≥ alendronate > risedronate), while non-NBPs lacked this effect. (b) Co-injection of clodronate or etidronate with zoledronate reduced these reactions, and also reduced the amount of zoledronate retained within the ear tissue. (c) When zoledronate and clodronate were intraperitoneally injected, clodronate hardly affected the ABRE of zoledronate (or those of other NBPs). In contrast, etidronate, when combined with zoledronate or another NBP, etidronate markedly reduced the ABRE of the NBP. Notably, etidronate reduced the ABRE of zoledronate even when it was injected 16 h after the zoledronate. Discussion: These results suggest that (i) clodronate and etidronate may inhibit the entry of NBPs into cells related to inflammation and/or necrosis, and thereby prevent the NBPs• f side effects, (ii) clodronate could be useful as a combination drug

with NBPs for preventing their side effects while preserving their ABREs, (iii) etidronate (but not clodronate) may competitively inhibit the binding of NBPs to bone hydroxyapatite, and this reagent may at least partly eliminate (or substitute for) NBPs that have already accumulated within bones, and (iv) etidronate, if used as a substitution drug for NBPs, may be effective at treating or preventing NBP-associated ONJ.

(1) Endo et al., Br J Pharmacol 1999;126:903-910.
(2) Monma et al., Calcif Tissue Int 2004;74:115-121.
(3) Funayama et al., Calcif Tissue Int 2005;76:448-457.

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OSTEOCLAST DEFECTS IN XLA PATIENTS ARE NEGATED BY THE LACK OF MATURE B CELLS AND ELEVATED INFLAMMATORY CYTOKINE PRODUCTION

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X-linked agammaglobulinemia (XLA) is caused by mutations in the gene for Bruton's tyrosine kinase (Btk), resulting in impaired B cell receptor signaling and maturational arrest of B cells. XLA patients have profoundly decreased peripheral B cells and serum immunoglobulins. Recent evidence in mice suggests that B cells play a role in normal bone physiology and maintenance of peak bone mass. Therefore we investigated the effect of the systemic absence of B cells on the bone density of XLA patients.

We utilized quantitative ultrasound (QUS) of the heel to investigate the BMD of ambulatory XLA patients (ages 36-53 years). In XLA patients and age-matched controls, serum markers of bone metabolism and inflammatory cytokine levels were quantified using ELISA. In order to assess the effects of B cell deficiency on osteoclast differentiation and activation in XLA, and to determine the role of activated B cells on osteoclastogenesis in vitro; TRAP positive multinucleated cell (MNC) formation and lacunar resorption assays were performed.

QUS showed that XLA patients, compared to age matched data, had significantly decreased bone density ($p=0.02$) and increased susceptibility for osteoporosis with advancing age. While no difference was found in the serum markers of bone metabolism, we found profoundly increased expression of IL-1 ($p=0.006$) and IL-6 ($p=0.0001$) in the serum of XLA patients compared to controls; these pro-inflammatory cytokines promote osteoclastogenesis. Lacunar resorption activity was defective in XLA patient osteoclast cultures, as previously described in murine Btk deficient studies. However there was a significant increase in TRAP MNC formation in XLA cultures compared to control cultures ($p=0.04$) . Depletion of B cells from control PBMC cultures resulted in significantly increased osteoclast differentiation ($p=0.02$). These results suggest that B cells secrete factor(s) that regulate osteoclastogenesis and are unlikely to involve OPG. Furthermore, B cell inhibition of osteoclastogenesis was significantly increased when B cells were pre-activated with LPS.

We provide unique evidence that human peripheral B cells secrete inhibitory factors of osteoclast differentiation and activation in vitro. Our novel finding of reduced bone density in XLA patients, suggest that absence of B cells in the peripheral blood, in combination with increased serum cytokine levels give rise to the overriding increase in osteoclast activity and decreased bone density in vivo.

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RANKL STORAGE IN SECRETORY LYSOSOMES IN OSTEOBLASTIC CELLS - THE INVOLVEMENT OF VPS33A

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Previous studies have indicated that the amount of receptor activator of NF-κB ligand (RANKL) expressed on the cell surface of osteoblastic cells is considered an important factor determining the extent of osteoclast activation. For example, the extracellular portion of RANKL is cleaved by matrix metalloproteinase 14 and a disintegrin and metalloproteinase 10, which are proteases localized on the plasma membrane, and suppression of these proteases results in increased osteoclastogenesis. On the other hand, osteoclast activation is inhibited by osteoprotegerin, which is a secreted decoy receptor for RANKL, and its deficiency causes severe osteoporosis. Based on these facts, it has been hypothesized that the number of RANKL molecules that are accessible to RANK is strictly regulated in order to maintain bone homeostasis in vivo. However, the mechanism of RANKL sorting to the cell surface is still unclear, and the mechanism needs to be thoroughly investigated in order to understand osteoclastogenesis in vivo. In the present

study, we have shown that RANKL is predominantly localized in lysosomal organelles, but little is found on in the cell surface of osteoblastic cells. We have also shown that RANKL is relocated to the plasma membrane in response to stimulation with RANK-Fc coated beads, indicating that the lysosomal organelles where RANKL is localized function as secretory lysosomes. In addition, using a protein pull-down method, we have identified vacuolar protein sorting (Vps) 33a, a component of the Class C Vps protein complex, as interacting with the cytoplasmic tail of RANKL. Furthermore, knockdown of Vps33a expression reduced the lysosomal storage of RANKL and caused the accumulation of newly synthesized RANKL in the Golgi apparatus, indicating that Vps33a is involved in transporting RANKL from the Golgi apparatus to secretory lysosomes. We have also shown that suppression of Vps33a affects the cell surface expression level of RANKL and disrupts the regulated behavior of RANKL. These results suggest that RANKL storage in secretory lysosomes is important to control osteoclast activation and to maintain bone homeostasis.

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RANKL SECRETION FROM SECRETORY LYSOSOMES IN OSTEOBLASTIC CELLS - THE INVOLVEMENT OF SMALL GTPASE RAB27A/B

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It has been established that Receptor Activator of NF-κB (RANK) Ligand (RANKL) expressed in osteoblasts induces osteoclast differentiation through binding to the receptor, RANK. This ligand-receptor interaction has been considered to occur by the cell-to-cell contact between osteoblasts and osteoclasts. On the other hand, our previous study revealed that RANKL is predominantly localized in secretory lysosomes but little in the plasma membrane. Therefore, the mechanism of RANKL translocation to the cell surface is considered important for osteoclastogenesis. By the analogy to the secretion mechanism of FasL, a member of TNF superfamily like RANKL, we examined the involvement of the small GTPase Rab27a in RANKL secretion in osteoblastic cell line, ST2 cells. To analyze the effect of Rab27a and other genes, we established stably knocking down ST2 cells using lenti virus encoding miRNA against target genes. When cell surface proteins were biotinylated, the amount of biotinylated RANKL was decreased by Rab27a suppression. We also performed coculture of stably knocked-down ST2 cells with bone marrow macrophages. TRAP activity, the marker of osteoclast maturation, was decreased by Rab27a suppression. These results suggest that Rab27a is involved in RANKL secretion in ST2 cells. We also examined the effect of Rab27b, the other isoform of Rab27 and Rab3a, closely-related to Rab27. Rab3a suppression did not alter the TRAP activity in the coculture, but Rab27b suppression decreased TRAP activity in coculture like Rab27a. These results suggest that Rab27a/b regulate RANKL secretion in osteoblastic cells. Next, we examined the effect of Rab27a/b on RANKL subcellular localization. While RANKL tagged with GFP were mostly localized in the secretory lysosomes in basal condition, the trend was observed by Rab27a/b suppression that RANKL slightly accumulated near the plasma membrane. This suggests that Rab27a/b are involved in the secretion of RANKL containing vesicles, especially in the tethering and/or fusion step of the vesicles with the plasma membrane. In conclusion, Rab27a/b are involved in RANKL secretion, and Rab27a/b suppression leads to the reduction in the ability to support osteoclastogenesis in osteoblastic cells.

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DOSE DEPENDENT ANABOLIC AND ANTI-CATABOLIC RESPONSE AFTER LOCAL ZOLEDRONATE TREATMENT OF CANCELLOUS BONE GRAFTS. A BONE CHAMBER STUDY IN RATS

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Bisphosphonates are strong inhibitors of bone resorption and normally administered systemically. Local application might be preferable in osteonecrosis, bone grafting or during callus formation. In some studies a decreased anabolic response has been noted. In the present study we evaluate how different doses, exposure time and modes of administration influence bone resorption as well as ingrowth of new bone into an allograft.

Cancellous bone grafts were harvested from male SD rats and randomized into five groups. The grafts were placed in a bone conduction chamber and inserted into the proximal tibia of 50 female rats. Zoledronic acid (0.5 mg/ml) was used for local graft treatment. The grafts were soaked in excessive zoledronate solution for 5 seconds and 10 minutes respectively before being rinsed in saline. In the third group, 8 µL of the solution was adsorbed to the graft without

rinsing. A saline group served as negative control and a systemically treated group as positive control. The grafts were harvested at 6 weeks and evaluated histomorphometrically.

The relative amount of remaining bone in the remodeled part of the graft bone (BV/TV) was used to evaluate bone resorption. BV/TV was 11% in the controls, 41% in the group receiving systemic treatment ($p=0.001$ vs control) and between 54-61% in the groups receiving local treatment ($p<0.007$ vs control and $p<0.01$ vs systemic).

The ingrowth distance of new bone into the chamber was used to evaluate the anabolic response and was 2.6 mm in the saline and 2.5 mm in the systemic group. In the 10 min group, a decreased ingrowth was found (1,6 mm, $p=0.007$ and $p=0.008$). In the other two local treatment groups, ingrowth was 2.3 mm (5 seconds soaking, n.s.) and 2.2 mm (no rinsing, n.s.)

We found a strong effect by a bisphosphonate on bone resorption but also a limited dose dependent inhibition of the ingrowth of new bone. Local treatment resulted in stronger inhibition of both resorption and bone formation compared to systemic treatment. Rinsing of the graft before implantation to remove any unbound zoledronic acid did not seem to make any marked difference.

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TROLOX PREVENTS OSTEOCLASTOGENESIS BY SUPPRESSING RANKL EXPRESSION AND SIGNALING

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Excessive receptor of NF- κB ligand (RANKL) signaling causes enhanced osteoclast formation and bone resorption. Downregulation of RANKL expression or its downstream signals may be a therapeutic approach to the treatment of pathological bone loss. In this study, we investigated the effects of Trolox, a water-soluble vitamin E analogue, on osteoclastogenesis and RANKL signaling. Trolox potently inhibited IL-1-induced osteoclast formation in bone marrow cell-osteoblast coculture by abrogating RANKL induction in osteoblasts. This RANKL reduction was attributed to the reduced-prostaglandin E2 production via downregulating cyclooxyganase-2 activity. We also found that Trolox inhibits osteoclast formation from bone marrow macrophages (BMMs) induced by M-CSF plus RANKL in a reversible manner. Trolox was effective only when present during the early stage of culture, implying that it targets early osteoclast precursors. Pretreatment with Trolox did not affect RANKL-induced early signaling pathways including MAPKs, NF- κB, and Akt. We found that Trolox downregulates RANKL induction of c-Fos protein by suppressing its translation. Ectopic overexpression of c-Fos rescued the Trolox inhibition of osteoclastogenesis in BMMs. Trolox also suppressed IL-1-induced osteoclast formation and bone loss in mouse calvarial bone. Taken together, our findings indicate that Trolox prevents osteoclast formation and bone loss by inhibiting both RANKL induction in osteoblasts and c-Fos expression in osteoclast precursors.

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CORNING BONE CELL ASSAY SURFACE

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Bone cell assays are very important component in drug discovery for bone related diseases such as osteoporosis, multiple myeloma, Paget's disease and cancer bone metastasis. A newly developed inorganic crystal coating on polystyrene cell culture plates has been shown to be suitable for various bone cell assays. These inorganic coating materials have similar physical and chemical properties to the inorganic phase of natural bone. The Corning Bone Cell Assay Surface has been evaluated for osteoclast differentiation and bone resorption, osteoblast differentiation, co-culture of osteoclast and osteoblast. Measurement of the in vitro inhibition of bone resorption by alendronate was also assayed on Corning Bone Assay Surface.

Corning Bone Cell Assay Surface provides: 1) a surface mimic bone inorganic phase; 2) a surface suitable to facilitate osteoclast differentiation and bone resorption, osteoblast differentiation and co-culture; and 3) a platform for bone related drug screening.

Category 6. Bone Formation, Cartilage and Bone Matrix

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KERATIN 18 IS OVER-EXPRESSED IN OSTEOBLASTS DERIVED FROM PAGETIC LESIONS BUT IS NOT INVOLVED IN THE FORMATION OF DISRUPTED MATRIX

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Paget's disease is a common focal bone disorder. Pagetic lesions, which result from over-activity of osteoclasts and osteoblasts, appear lytic at early stages and later turn sclerotic, with areas of irregular, disorganised bone matrix. Pagetic osteoclasts are grossly abnormal, and have been the focus of most of the research on the cellular mechanisms of Paget's. Given the tight coupling between osteoclasts and osteoblasts, we characterised changes in the pagetic osteoblast that could contribute to the development of the disease. We compared gene expression in osteoblasts and bone marrow cells from pagetic and non-pagetic patients. Microarray analysis identified a number of differentially regulated genes, and the intermediate filament protein keratin 18 (KRT18) was one of the most highly upregulated genes in pagetic osteoblasts. Real-time RT-PCR comparing 28 pagetic samples to 49 controls confirmed that KRT18 expression is more than three times higher in pagetic cells. In the present study we investigated the effects of KRT18 over-expression on osteoblasts and mesenchymal cells.

Primary human osteoblasts were transduced with a KRT18 adenoviral vector and compared to cells transduced with a control vector. Real-time RT-PCR showed that KRT18 over-expressing cells have increased levels of interleukin 1β, Dkk1, and the chemokine MCP1, genes that had previously been identified as up-regulated in pagetic osteoblasts. In order to study the possible effects of KRT18 over-expression on cell morphology and extracellular matrix formation, we cultured virally transduced human primary mesenchymal cells in 3-dimensional collagen scaffolds. The scaffolds provide an extracellular structure which resembles the bone environment and is therefore better suited for the study of cell morphology and matrix formation and mineralisation. Cells were analysed using confocal imaging. Cells were also stained for alkaline phosphatase activity and calcium deposition was measured by alizarin red staining. Cultures of KRT18 over-expressing cells were not significantly different from the controls.

In conclusion, these results suggest that KRT18 plays a role in osteoblast biology, and overexpression of this gene can reproduce some of the features of pagetic osteoblasts, however, this does not appear to include the disrupted matrix formation.

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EPHB/EPHRIN-B INTERACTIONS ASSIST IN MSC NICHE MAINTENANCE AND CONTRIBUTE TO BONE REMODELLING FOLLOWING INJURY

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The bone marrow contains mesenchymal stromal/stem cells (MSCs) that reside within a perivascular niche. This precursor population is essential for regulating skeletal tissue homeostasis, including bone formation and repair. Bone remodelling is mediated by bone forming osteoblasts derived from MSC, and by bone resorbing osteoclasts that originate from the haematopoietic lineage. However, the molecular signals that maintain multi-potential MSC populations within the stem cell niche and the mechanisms that drive their mobilization towards the bone surfaces to facilitate bone formation are not well defined. The Eph/ephrin family of receptor tyrosine kinases have been implicated in the maintenance of stem cell niches including neural, intestinal and dental tissue, and more recently in the regulation of bone homeostasis. We have characterized the gene and protein expression of EphB/ephrin-B molecules on human culture expanded MSC populations and found that the EphB1, B2 and B4 receptors and ephrin-B1/B2 ligand were the highest expressed B class Eph/ephrin molecules. Functional studies using Fc fusion proteins bound to the extracellular portion of EphB2 or ephrin-B1 showed that reverse ephrin-B signalling inhibited human MSC attachment

and spreading, while forward EphB signalling promoted MSC migration. Furthermore, EphB expressing MSC were found to restrict their capacity for self-renewal while activated ephrin-B expressing MSC promoted osteogenic differentiation. A murine femoral fracture model was used to elucidate the contribution of EphB/ephrin-B molecules to skeletal tissue repair and bone remodelling. Gene expression for both EphB and ephrin-B significantly increased during the early stage of bone repair and returned to steady-state levels at the later stages of bone remodelling. EphB2 and ephrin-B1 were found to be most abundantly expressed within the callus site, including blood vessels, hypertrophic and calcified chondrocytes, osteoblasts, osteocytes, and newly forming bone. In this study we have demonstrated the role of EphB/ephrin-B family members in human MSC self-renewal, migration and osteogenic differentiation, and appear to be differentially expressed in skeletal tissues during bone fracture healing using a murine femoral fracture model. These observations suggest that EphB/ephrin-B interaction potentially maintain human MSC within their niche under steady state conditions, while promoting migration and skeletal tissue homeostasis following injury.

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RECEPTOR ACTIVATOR NF-KAPPAB LIGAND (RANKL) EXPRESSION IN SYNOVIAL TISSUE (ST) AND BONE MINERAL DENSITY (BMD) IN RHEUMATOID ARTHRITIS (RA) PATIENTS

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Background: RA is commonly characterized by localized & generalized bone loss. Excessive release of cytokines and growth factors due to inflammation associated with the effect of antirheumatic therapy (mainly corticosteroids) significantly influence total bone mass. Recent evidence indicates that RANKL has a pivotal role in osteoclastogenesis and inflammation-induced bone destruction in RA.

Purpose: To compare RANKL expression in the Synovial Tissue (ST) from patients with active and inactive RA and from osteoarthritis patients and to investigate the relationship between RANKL levels and BMD.

Methods: 26 patients with RA, were recruited and clinical status was scored using the DAS28. The control group included 22 matched osteoarthritis patients. All RA patients were on immunosuppressive agents, but none of them received corticosteroids or TNF-α antagonists. Immunohistological analysis of ST biopsy specimens was performed according to the Avidin-Biotin-Complex method, using a monoclonal antibody to detect RANKL. Sections were evaluated by semiquantitative analysis to compare RANKL expression between groups.

Results: Higher levels of RANKL were expressed in ST from patients with active RA than in ST from patients with inactive RA and osteoarthritis. DXA measurements revealed that all patients with RA showed significant decreases in BMD compared with controls. A comparison of BMD between patients with active and inactive disease did not reveal a significant effect of clinical disease activity on the lumbar spine and total proximal femur BMD. No relation was found between the lumbar and femoral BMD and the expression of RANKL in ST in patients with inactive RA. However, a good correlation was found between the decrease in BMD and the increase in RANKL expression in ST from patients with active disease.

Conclusion: The highest expression of RANKL was detected in RA patients with active synovitis. Our data showed a significant correlation between the increased RANKL expression in ST from RA patients with active disease and the decrease in BMD, suggesting that RANKL may have a role in generalized bone loss as well. Normal level of RANKL in the ST of patients with inactive disease at the time of synovial biopsy raise de possibility that effective treatment of the disease m

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CAN SERUM MARKERS OF BONE METABOLISM PREDICT THE PROGRESSION OF KNEE OA?

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Introduction: Cartilage and bone are affected simultaneously as osteoarthritis progresses, but this relationship is incompletely understood. This study aimed to examine the relationship between serum markers of bone formation and resorption, and change in cartilage quantity over 2 years, and to determine whether ratios of markers of bone formation to resorption provide additional information.

Methods: Change in cartilage volume over 2 years was measured in 117 subjects with symptomatic knee osteoarthritis using MRI. The relationships between change in cartilage volume and baseline levels of serum markers of bone formation (intact N-terminal propeptide of type I procollagen (PINP) and osteocalcin), resorption (N-telopeptide of type I collagen (NTX-I), the C-telopeptide of type I collagen (CTX-I) and the C-telopeptide of type I collagen (ICTP)) and ratios of markers of bone formation to resorption were examined.

Results: Individually, lower levels of PINP ($p = 0.02$), osteocalcin ($p = 0.01$), NTX-I ($p = 0.02$), and CTX-I ($p = 0.02$) were associated with elevated medial cartilage volume loss. No significant associations were obtained between marker ratios of bone metabolism and change in cartilage volume.

Conclusions: The results of this study suggest that overall, lower serum levels of bone metabolism in osteoarthritis may be predictive of cartilage loss. Further insight into understanding how individual markers of bone metabolism affect cartilage loss may facilitate understanding of the pathogenesis of this disease and aid in predicting disease progression.

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ASSOCIATION BETWEEN PRE-EXISTING MICRODAMAGE, COLLAGEN CROSS-LINKS, BONE VOLUME FRACTION AND COMPRESSIVE MECHANICAL PROPERTIES OF HUMAN VERTEBRAL TRABECULAR BONE

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Studies have suggested that age-related increases in fatigue microdamage and changes in the profile of collagen crosslink concentration may contribute to skeletal fragility. To further explore this notion, we determined the influence of pre-existing microdamage, collagen cross-link concentration and bone volume fraction (BV/TV) on the mechanical behavior of human trabecular bone. To do this we removed two cylindrical trabecular specimens (8.2 mm diam, 10 mm height) from L2 vertebrae of 52 recently deceased donors (54 – 93 yrs of age; 22 men and 30 women). One core was prepared for histologic analysis of pre-existing microdamage by bulk staining in xylenol, whereas the other core was used for assessment of 3D morphology by microCT and compressive mechanical properties. The central part of the vertebral body, containing exclusively trabecular bone, was retained for analysis of pyridinoline (PYD), deoxypyridinoline (DPD) and pentosidine (PEN) content by high performance liquid chromatography. Non-parametric tests were used (Spearman coefficient correlation r'). Linear crack density (#/mm^2) and diffuse damage area density (%) increased with age ($r' = 0.36$, $p = 0.01$, $r' = 0.32$, $p=0.022$, respectively), as did the PYD/DPD ratio ($r' = 0.31$, $p = 0.028$). Elastic modulus and ultimate strength decreased with age ($r' = -0.36$, $p = 0.01$, $r' = -0.39$, $p=0.01$, respectively), whereas post-yield strain was unchanged. BV/TV was strongly correlated with elastic modulus ($r' = 0.66$, $p<0.001$) and ultimate strength ($r' = 0.74$, $p<0.001$). The elastic modulus was weakly correlated to mean crack length ($r' = 0.30$, $p = 0.036$), but otherwise not associated with pre-existing microdamage. Collagen cross-link concentrations were not associated with mechanical properties. PYD concentration was positively correlated to linear microcrack density ($r' = 0.33$, $p = 0.017$) and to total microcrack density ($r' = 0.36$, $p = 0.008$), whereas PYD and DPD were negatively correlated to mean microcrack length ($r' = -0.30$, $p = 0.029$ and $r' = -0.28$, $p=0.046$, respectively). PEN concentration was not associated with microdamage. In summary, this study of trabecular bone from elderly human donors indicates limited influence of microdamage burden or collagen cross-link concentration on trabecular bone mechanical properties. These data suggest that bone volume fraction is the predominant determinant of trabecular bone mechanical properties.

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WHICH SUBCHONDRAL BONE MEASURE IS THE BEST PREDICTOR OF CARTILAGE DAMAGE?

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Background: It is widely accepted that subchondral bone plays a central role in the pathogenesis of osteoarthritis (OA). Evidence suggests that subchondral bone changes precede cartilage damage, indicating bone changes may be an early stage of OA. The objective of this study was to describe the association of baseline areal and volumetric knee subchondral bone mineral density (sBMD), tibial bone size, and bone marrow lesions with knee cartilage defect progression and cartilage volume loss in adult males and females.

Methods: A total of 390 subjects (mean age 63 years, range 51-79) were measured at baseline and approximately 2.5 years later. Knee cartilage volume, cartilage defects (range, 0-4), and bone size were determined using T1-weighted fat saturation MRI. Bone marrow lesions (range, 0-3) were determined using T2-weighted MRI images. Areal sBMD was assessed by DXA. Volumetric sBMD was calculated as: bone mineral content/(bone area × region of interest).

Results: For the medial compartment, baseline bone size was positively associated with increases in tibiofemoral cartilage defects (odds ratio [OR] 2.6 per change in SD, $P < 0.01$) and negatively associated with annual cartilage volume change ($\beta = - 0.01$, $P < 0.01$). Baseline tibial and femoral bone marrow lesions were positively associated with tibial and femoral cartilage defect increases (OR 1.7 per grade, $P = 0.01$; OR 2.3 per grade, $P < 0.01$, respectively) and negatively associated with compartment specific cartilage volume change ($\beta = -1.0$, $P = 0.024$; $\beta = -2.5$, $P < 0.01$, respectively). Similar results were observed for the lateral compartment. Neither, areal or volumetric sBMD at baseline were associated with increases in cartilage defects or cartilage volume loss.

Conclusions: Both tibial bone area and bone marrow lesions at baseline predict compartment specific cartilage defect progression and cartilage volume loss. The lack of association between sBMD and cartilage damage suggests that tibial bone size and bone marrow lesions may be the preferred subchondral bone targets for intervention.

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ANTI TUMOR NECROTIC FACTOR AGENTS PROMOTE THE ABILITY OF THE BMP-2 INDUCED ECTOPIC BONE FORMATION

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Tumor necrosis factor-α (TNF-α) plays key role in the regulation of inflammatory synovitis and the subsequent destruction of cartilage and bone in rheumatoid arthritis (RA). Etanercept (ETN), which is a recombinant human soluble TNF receptor and inhibits TNF action, is effective in the treatment of RA. We investigated the effect ETN on rhBMP-2 induced ectopic bone formation in vivo. A block copolymer composed of poly-D, L-lactic acid with random insertion of p-dioxanone and polyethylene glycol (PLA-DX-PEG polymer) was used as the delivery system. Each polymer disc (6mm,30mg) containing 5μg rhBMP-2 were implanted into the left dorsal muscle pouch of mice (n=5). ETN were subcutaneously injected (25mg/human=12.5μg/mouse) twice per week in a dose-dependent manner (group1;placebo, group2;12.5x1/1000,group3;12.5x1/10, group4;12.5, group5;12.5x10) as systemic administration groups, whereas the single dose of ETN were also embedded in each polymer with rhBMP-2 in the same manner as a local administration groups. All implants were increased radiodensity at 3 weeks posttransplantation ,consistent with a significant increase of bone mineral contents (BMC) of ossicles. Bone histomorphology exhibited a significant increase in both trabecular BV/TV and osteoblast number and a significant decrease in osteoclast number dose-dependently both systemic and local administration of ETN. No significant difference in body weight and serum data (AlP,Ca,P) among groups was observed during the experimental period. These data suggested that the optimal dose of ETN systemically or locally enhanced the bone inducing capacity of rhBMP-2 with no apparent systemic adverse effect.

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HUMAN VERSUS SHEEP VERTEBRAL CORTICAL BONE STRAIN AND INTERVERTEBRAL DISC MECHANICAL PROPERTIES

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INTRODUCTION: Animal models are increasingly being used for research studies of the spine, and the sheep lumbar motion segment has been shown to have morphological and biochemical similarities to human segments. However, the differences in vertebral cortical bone principal strains and disc mechanical (stiffness and phase angle) properties have not been quantified.

METHODS: Twenty, lumbar disc segments from 14 human spines and six Merino wethers were obtained. Strain gauge rosettes were bonded to the vertebral cortical bone surface of the inferior, disc-endplate boundary at three locations: right/left lateral and anterior. Each disc was equilibrated under an 0.2 MPa compressive preload in a 0.15M PBS bath and subjected to 10 sinusoidal cycles in compression (1 MPa) at 0.5 Hz. Principal strains for each rosette, stiffness and phase angle were calculated and unpaired t-tests were conducted to identify significant interspecies differences.

RESULTS: No significant differences were present between maximum or minimum principal strains at each cortical location for human or sheep ($P>0.82$), allowing for pooling of strains. Human minimum cortical principal strains were of significantly larger magnitude than for sheep (-578±400 με versus -74±49 με, $P<0.001$). Maximum human principal strains (205±108με) were not significantly different to sheep (87±50με, $P=0.055$). Significant differences in compressive stiffness were found between human (2,497±569 N/mm) and sheep (1,473±394 N/mm) discs. No differences were found for phase angle between human (4.9±0.4°) and sheep (5.1±0.3°) discs ($P>0.5$).

DISCUSSION: Cortical bone strains, particularly minimum principal strain were substantially larger in human bone, suggesting stiffer cortices in the sheep. Vertebral cortical bone density (BV/TV) has been measured as approximately 41% in sheep [1] and approximately 11% in humans [2], with this difference probably contributing to the stiffer minimum principal cortical strains measured in sheep. No data could be found on the cortical thickness in sheep, however, the higher sheep BV/TV suggests that it is likely to be greater than that of human cortical thickness (mean±SD: 0.52±0.35mm [3]), and consistent with the observations in goats demonstrating a higher trabecular density compared to human vertebrae [4].

These important differences should be taken into account when using a sheep model to assess disc and bone mechanical properties.

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(2) Hulme PA, et al., Bone 41(6):946-57, 2007.
(3) Fazzalari NL, et al., Joint Bone Spine 73(3):293-7, 2006.
(4) Smit TH, Eur Spine J 11(2):137–144, 2002.

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BONE QUALITY IN VERTEBRAL FRACTURE: MICRODAMAGE, MINERALISATION AND MICROARCHITECTURE OF THE CORTICAL SHELL IN LUMBAR VERTEBRAE.

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Resistance to vertebral fracture is provided by a bone structure that integrates the cortical shell, or cortex, with trabecular bone. The cortical shell of vertebral bodies demonstrates a contribution to load sharing that is disproportionate to its relative bone volume fraction, but there is a paucity of data on the bone quality of the cortex relative to the adjacent trabeculae. The aim of this project was to quantify the microdamage burden, mineralisation and microarchitecture of the cortical shell of the human L2 vertebra. Vertebrae from 22 donors, 54 to 93 years (8 males and 15 females) were bisected and bulk stained in 0.5 mM xylenol orange. The anterior and antero-lateral cortex of the right side was embedded in plastic and scanned with a microCT (Skyskan 1076, Lyon) with voxel size at 18 um, then thick sections (80-100 μm) were obtained for analysis of microdamage, thin sections for histomorphometry and a 100 μm section for analysis of the degree of mineralisation (quantitative microradiography) and microhardness. Contrary to our hypothesis, the mean degree of mineralisation (DMB) and the Vickers micro-hardness (Hv) of the cortical bone were significantly lower than that of the adjacent trabeculae, and its mineral heterogeneity index (HI) higher ($p < 0.01$).

In the superior region, close to the endplates, microcrack density was greater in the trabeculae, than in the cortex (N/mm^2)($P < 0.05$); but the cortex had significantly higher volume of diffuse damage (DxV/BV) in both the superior and middle regions than trabeculae ($P < 0.05$). The difference between cortical and trabecular bone mineralisation is not evident for the iliac crest (Boivin et al., 2005, 2008) which also has a relatively low microdamage (Mdx) burden (Chapurlat et al, 2007). These differences strongly suggests that remodelling in the cortical bone of vertebral bodies is higher than that of the trabeculae, which could be a result of load sharing by the cortex and its associated Mdx burden.

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(2) Boivin et al., Bone 43(3):532-8, 2008.
(3) Chapurlat et al., JBMR 22(10):1502-9, 2007.

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ENHANCED BONE INGROWTH ONTO RHBMP-2-COATED POROUS TITANIUM IMPLANT PLACED IN THE DISTAL FEMUR OF RABBITS

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Introduction: Implants with various surface structures are currently used without bone cement to obtain secure fixation to bone. Initial fixation of the implants to bone is achieved within several weeks via new bone formation at the bone/ implant interface. Therefore, faster and more extensive growth of new bone with use of bone-inducing agents will promote tight implant/bone integration. In this study, we attempted to use recombinant bone morphogenetic protein-2 and a system for local delivery of it in combination with porous surfaced implants to examine the usefulness of BMP-2 for rapid and secure fixation of the implant placed in distal medullary canal of the rabbit femur, and evaluated the tightness of fixation of the rhBMP-2-coated porous titanium implant in the bone marrow.

Materials and Methods: A porous plasma sprayed titanium cylindrical implant with a diameter of 6mm and length of 10mm was placed in the bone marrow of the distal femur of New Zealand white rabbits (1-2 years of age). Forty rabbits were divided into 5 groups (n=8 each). β -tricalcium phosphate (β TCP) powder (60mg), a polymer gel (PLA-PEG copolymer; 60mg), or rhBMP-2 (15, 30, or 60 μ g) was mixed and pasted onto the implants. The distal femurs with implants were harvested at 3 or 6 weeks after surgery and examined by soft x-ray, determination of bone mineral density (BMD) around the implant surface, biomechanical testing (push out test), and histology.

Results: Determination of BMD and biomechanical testing at 3 weeks revealed significantly tighter implant fixation in the rhBMP-2 (15 and 30 μ g) groups than in the control group ($p<0.01$), though no significant difference between the rhBMP-2 (60 μ g) and control group was noted (Fig.1). Histological and BMD analyses demonstrated more new bone on implants in the rhBMP-2 (30 μ g) group than in the other groups.

Conclusion: Our findings demonstrate that rhBMP-2 coating promotes initial biomechanical fixation of implants with bone in the bone marrow. The appropriate concentration of rhBMP-2 for implant fixation in bone marrow remains to be determined.

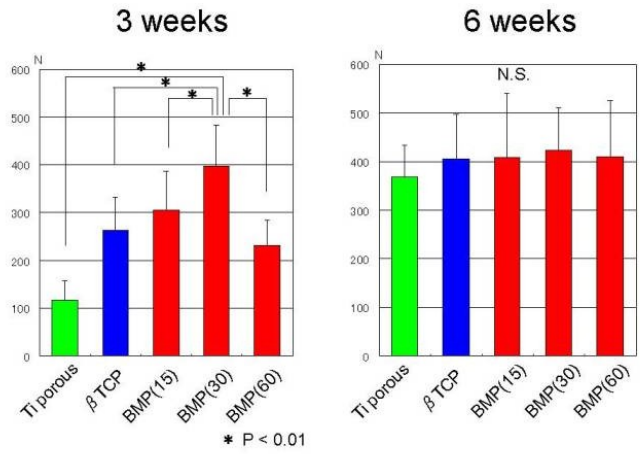

Fig.1 biomechanical test

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THE ROLE OF PROTEASE-ACTIVATED RECEPTOR-2 IN SKELETAL GROWTH AND BONE REPAIR

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The G-protein-coupled receptor, protease-activated receptor-2 (PAR-2), has been implicated in physiological responses and disease states of the cardiovascular, gastrointestinal, respiratory and musculoskeletal systems. PAR-2 is expressed by osteoblasts and activated by proteases including coagulation factor Xa, which is likely to be present in bone repair sites. The aim of the current study was to investigate the role of PAR-2 in skeletal growth and bone repair using wild type (WT) and PAR-2 knock out (KO) mice. Tibiae were isolated from male and female, WT and KO mice (n=6) at 50 and 90 days of age and were analysed using Micro-CT (10 μm resolution). At 50 days the midshaft of the tibia of KO mice exhibited significantly increased cortical thickness, cortical bone area and total cross-sectional area in both males and females. At 90 days none of the above parameters were significantly different between WT and KO mice except total and medullary cross-sectional area, which were reduced in KO males. To identify the role of PAR-2 in bone repair a cortical defect was made in tibiae of WT and KO mice by drilling a hole (500 μm diameter). The progression of bone repair in WT and KO mice was analysed using Micro-CT. Seven days post surgery, there was significantly less new bone in the drill site in PAR-2 KO mice compared to WT. Functional responses of primary calvarial osteoblasts from WT and KO animals were studied in vitro. Increased osteoblast survival and reduced apoptosis were observed in osteoblasts isolated from KO neonatal mice compared to their WT counterparts. However there was no difference in alkaline phosphatase (ALP) activity or the ability to form mineralised nodules. Bone marrow cultures from KO mice showed fewer ALP-positive colony-forming units and osteoclasts compared to WT counterparts. In primary osteoblast cultures, FGF-2, thrombin and TNF-alpha, which are important in bone repair, significantly up-regulated PAR-2 mRNA expression. These results suggest that PAR-2 activation contributes to the determination of cells of the osteoblast and osteoclast lineages within bone marrow, and thereby participates in the regulation of skeletal growth and bone repair.

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THE ROLE OF TWIST-1 AND -2 IN THE MAINTENANCE OF MESENCHYMAL STROMAL/ STEM CELLS

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Bone marrow mesenchymal stromal/stem cells (MSCs) have been shown to possess the potential to differentiate into a variety of different stromal cell types including bone, fat and cartilage, and other lineages such as muscle cells and astrocytes. The efficacy of ex vivo expanded MSC preparations as therapeutic agents in different regenerative medicine applications has also been demonstrated in pre-clinical and clinical trials. However, only a minor proportion of MSC clonal cell lines can maintain a primitive multi-potential phenotype following *ex vivo* expansion. Moreover, during subculture the progeny of clonogenic MSC exhibit a diminished capacity to undergo cellular division and multi-differentiation. This has hampered the use of MSCs in the development of cellular therapies, in particular for skeletal tissue regeneration. We have recently found that purified populations of STRO-1^{bright}/$CD106^{+}$ or STRO-1^{bright}/$CD146^{+}$ selected human MSC express high levels of the basic helix-loop-helix transcription factors, Twist-1 (Twist) and Twist-2 (Dermo-1) which are subsequently down regulated during *ex vivo* expansion. Retroviral mediated enforced expression of both Twist-1 and -2 dramatically increased the proliferation rates and lifespan of cultured human MSCs. Twist-1 and -2 overexpressing MSC were also found to express an immature phenotype associated with elevated levels of the stromal precursor marker, STRO-1. Furthermore, Twist-1 and -2 overexpressing MSC lines were inhibited in their capacity to undergo osteochondrogenic development both *in vitro and in vivo*. However, enforced expression of both Twist-1 and -2 showed no effect on the capacity of these lines to undergo adipogenesis. These data imply that the Twist family of transcription factors known to be important in mesodermal development also play a role in the maintenance and growth of immature MSC populations. The present study lays the foundation for developing future strategies to direct and enhance the growth and developmental of culture expanded MSC for tissue engineering applications.

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TIMING OF STIMULATION OF ENDOGENOUS BMP2 EXPRESSION IN FRACTURE REPAIR

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BMP2 is a major growth regulatory factor involved in fracture repair (1). The rationale for administering BMP2 locally at the fracture site is to enhance the fracture healing process but there is not a clear agreement as to when BMP2 should be administered. Local administration of exogenous BMP2 suggests that giving the agent some time after fracture may produce the best effects (2,3). In this study, small molecule mimics of BMP2 were used to induce endogenous BMP2 expression at the fracture site and to determine if the effect on fracture healing acceleration was enhanced when the compounds were administered 1-2 weeks after fracture (delayed treatment (DT)) in a commonly used preclinical model of fracture repair in rats.

Lovastatin (LV) has been shown to be effective for fracture repair when injected locally immediately after fracture (4). A single dose of the drug was administered after the fracture was created (day 0) or one week later (day 7). Radiographically, fractured femurs showed enhanced repair at 4 weeks with LV DT with almost complete healing and small callus present while large callus and incomplete bridging was seen in the control group. DT produced >40% increase (15 ug) in maximal breaking force and structural stiffness while no increase was noted at this dose with treatment at day 0. Using a high resolution uCT, increase in cortex bridging was observed in DT with good correlation between uCT measurements and callus strength.

On a separate experiment using the same model, rats were treated with a local injection of a peptidyl aldehyde proteasome inhibitor (PSI) (American Peptide Co). Radiographic fusion score was higher in all groups treated with PS1 at 2 weeks and 4 weeks than in controls . Callus BMD measured by uCT and biomechanical strength was also increased by PS1 treatment, and best enhanced in DT group .

Conclusions:

· Local administration of small molecular mimics of BMP2 enhance fracture repair and delayed treatment of these compounds seems to increase their effectiveness.

· The use of these compounds may be promising not only for fracture repair but for other diseases where BMP2 has proven to be effective.

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NOVEL LOCALIZED AND RELEASE-CONTROLLED RHBMP-7/RHOP-1 CORE-SHELL NANOPARTICLES ACCELERATE TIBIAL DISTRACTION OSTEOGENESIS IN RABBITS

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The requirement for supra-physiological and expensive dosages of recombinant bone morphogenetic proteins (rhBMPs) in the milligram range is still necessary for satisfactory bone healing. Localized and release-controlled carriers/delivery systems for the expression of the biologic potency of rhBMPs according to defect anatomic site, size and vascularity are thus essential.

In an initial *in vitro* study, we have successfully encapsulated a model protein, bovine serum albumin in monodisperse and non-toxic nanoparticles constituting a core of cationic liposomes and a shell constructed through the layer-by-layer assembly (via electrostatic interactions) of alternating layers of natural polymers; anionic alginate and cationic chitosan. The release profile was characterized by an initial burst followed by sustained albumin release, highly

desirable for growth factor delivery; particularly in large bony defects. We then investigated the ability of the core-shell nanoparticulate delivery system to encapsulate a range of concentrations of BMP-7 (also known as osteogenic protein 1 or OP-1) for the potential administration via a parental injection as is preferable by surgeons. The system exhibits high physical stability in simulated physiological media as well as an extended shelf-life allowing for immediate protein loading prior to administration, preventing degradation or loss of the entrapped growth factor. The nanoparticles offer copious compartments for protein entrapment including the aqueous core and within the customizable polyelectrolyte layers in the shell. A sustained triphasic linear release of BMP-7 was evident for an extended period of 45 days with the bioactivity of the protein maintained via enhancing pre-osteoblast differentiation.

In a rabbit model of tibial distraction osteogenesis, we demonstrate that osteogenesis and consolidation are accelerated via a single injection of the core-shell delivery system loaded with a dose of no more than 1.0 microgram (ug) rhBMP-7/rhOP-1 in comparison to earlier results from a single injection of rhBMP-7/rhOP-1 (75.0 ug in saline), accentuating the role of our novel local and release-controlled core-shell nanoparticles.

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INCREASED BONE MATRIX MINERALIZATION IN SCHNURRI-3 NULL MICE

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Schnurri-3 (Shn3) controls the degradation of Runx2 and is a potent and essential regulator of postnatal skeletal remodeling. While Shn3 null mice show a dramatic increase in bone mass, no data are available that examine whether the bone matrix is altered in any way. To assess the bone material quality, we used quantitative backscattered electron imaging (qBEI), Scanning small-angle X-ray scattering (scanning-SAXS) and Fourier transform infrared imaging (FTIRI). T rabecular and cortical bone of the distal femur of Shn3 null and wildtype (wt) mice were investigated at the ages of 6 weeks, 4 months and 18 months.

qBEI analysis revealed no difference between wt and Shn3 mice in the degree of mineralization (CaMean), heterogeneity of bone mineralization (CaWidth) or amount of low mineralized bone (CaLow) in trabecular bone of the distal femur at 6 weeks of age, despite the 7-fold increase in BFR in Shn3 null mice. CaMean, CaWidth as well as amount at CaLow remained constant in wt mice with age. At 4- and 18 months of age, there was a significantly higher CaMean and a significantly lower CaLow in Shn3 null mice when compared to similar data at the age of 6 weeks. Similar changes were observed in cortical bone.

The results of the FTIRI analysis indicated a higher collagen crosslink ratio in the wt-groups compared to the shn3-group, evident in the primary mineralization bone areas. The results in the forming areas showed a decrease in formation of nonreducible, mature cross-links (pyr) in the shn3 group. No significant differences between the wt and shn3 groups were observed in this ratio when areas of secondary mineralization were compared.

Using SAXS, no significant differences in crystal size and orientation were found in Shn3 null mice.

Our data indicate an acceleration of the processes of bone matrix mineralization as well as collagen maturation in shn3 null mice when compared to wt animals.

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CELL SHAPE CHANGE AND ACTIN CYTOSKELETON REMODELING DRIVES HUMAN TENOCYTES TOWARDS A CHONDROCYTIC PHENOTYPE

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Human tendon undergoes chondroplastic metaplasia when exposed to compressive loading and during chronic degeneration. This phenotypic alteration reverses when compressive loading is removed but is currently irreversible in common and disabling degenerate conditions such as rotator cuff tear. Since there is no overt compression in most rotator cuff tendon tears we hypothesize that release of tension due to tendon tearing as well as altered local tension due to matrix degeneration may both contribute towards altered cell shape. Our group and others have found that

agents capable of altering cell shape via remodeling the actin cytokeleton promote chondrocyte marker expression in chondrocytes. We find that lovastatin, via effects on prenylation of small GTP binding proteins, is able to enhance expression of glycosaminoglycan and collagen type II in both adult bovine primary chondrocytes and the murine ATDC5 cell line. We have therefore altered cell shape and induced actin cytoskeleton remodeling in healthy human primary hamstring tenocytes. The effect of both classical (cytochalasin D) and novel (ROCK inhibitor Y27632, c-src inhibitor PP2, lovastatin) cytoskeletal remodellers have been tested by assessing their effect on tenocyte morphology (rhodamine phalloidin staining), immunocytochemistry and gene expression patterns (rtq-PCR). Human tenocytes in culture express stable levels of key phenotypic markers such as the putative master tenocyte transcription factor, scleraxis, for up to 5 passages. Scleraxis levels drop by passage 8 and we have therefore restricted our study to passages 1-3. Cytochalasin D and lovastatin both immediately affect actin arrangement and upregulate Sox 9 expression in tenocytes at 24, 48 and 72h of treatment. PP2 significantly upregulates Sox 9 at 72h. Longer treatment over 1 week with Lovastatin doses from 0.2 to 2uM upregulates Sox 5 (up to 4.7-fold) and Sox 6 (up to 5.5 fold) as well as Sox 9 (2.4 fold). Tendon normally expresses collagen types I and III, with undetectable levels of collagen type II. However, we detect collagen type II in the degenerate rotator cuff and have therefore subjected healthy tenocytes in culture to 3 weeks of exposure to the panel of cytoskeletal inhibitors. Only lovastatin induces de novo expression of collagen type II in tenocytes after this prolonged exposure. This provides novel insight into the physiology of tendon and suggests a potential mechanism for the phenotypic shift of tenocytes during degenerative disease.

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ACTIN CYTOSKELETON AND B-CATENIN LOCALIZATION IN OSTEOGENIC CELLS OF MOUSE CRANIAL SUTURE UNDER TENSILE STRESS

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Mechanical stress stimulates differentiation of osteoblasts and bone formation. Cytoskeleton and cell adhesion molecules have been shown to be one of the important factors for mechanotransduction. To clarify the role of these molecules in this mechanism, the changes of the distribution of F-actin and β-catenin in tensile-stressed mouse cranial suture cells were investigated. Tensile stress was applied to the sagittal sutures of 4-day-old mice calvariae in culture. After 1, 3 and 6 hrs, the specimens were fixed, and stained with Alexa 488- phalloidin and antibody to β-catenin. They were observed with confocal laser scanning microscope. As a control, non-treated calvariae were fixed immediately after dissection and processed in the same way. The osteoblasts located near the parietal bone edges of control specimens were oval-shaped, in which F-actin was mainly located throughout their cell-cortices, under the cell membrane. Beta-catenin was also located in the cell-cortex, but did not thoroughly underline the cell membrane. In these controls, fibroblastic cells located near the center of the suture had less F-actin at the cell-cortices, and were accompanied by a random network of actin fibers. In the fibrous periosteum layer, fibroblastic cells had a great number of actin fibers. In these fibroblastic cells, β-catenin was observed as small dots. The sutures of the stressed specimens were expanded in width. In the 3-hr stressed suture, the osteoblasts were elongated in the direction of the tension, and the F-actin and β-catenin located in their cell-cortices became denser. In the 6-hr stressed suture, most of the cells were further elongated and preosteoblastic cells were increased in number in the area between the osteoblasts and the fibroblastic cells of the center of the suture. These preosteoblastic cells had both thick actin layer in the cell-cortices and actin fibers which aligned in the direction of the tension. Beta-catenin was also observed in the cell-cortex. These results show that F-actin and β-catenin are located in the cell-cortex in mature osteoblasts, and these molecules are becoming denser with tensile stress. This structural change of cell-cortex suggests the adaptation of the osteoblastic cells to the applied mechanical stress.

A 3D CULTURE MODEL FOR PRIMARY ADULT HUMAN OSTEOBLASTS

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INTRODUCTION: A functional relationship between the inhibition of proliferation and induction of genes associated with matrix maturation for in vitro osteoblast culture was suggested by Owen et al. (1990). The monolayer culture of primary human osteoblasts often shows unsatisfying results for cell differentiation, matrix deposition and calcification. We aim to introduce a more physiological culture model for primary human osteoblasts by rapid cell differentiation and matrix development.

MATERIALS & METHODS: Primary human osteoblasts were used for pellet cultures seeding 36000 cells per pellet. Pellet formation was achieved by centrifugation at 500xG (10min). Cell viability was determined by lactate dehydrogenase (LDH) assay. DNA quantification, alkaline phosphatase (ALP) activity, and mineral apposition by Alizarin staining were performed. Comparative monolayer cultures were seeded using the same cells.

RESULTS: In contrary to 2D culture, the cell number within osteoblasts cell pellets slightly decreased over 27d [Fig.1A]. LDH activity demonstrated uniform cell viability throughout the individual pellets [Fig.2A]. Pellet cell morphology showed a cuboidal central cell population surrounded by flattened cells on the pellet surface. While monolayer ALP activity peaked by 7d, the maximum ALP activity in the pellets was detected by 3d [Fig.1B]. Immunohistological ALP labelling revealed concentrated activity areas throughout the whole of the cell pellets [Fig.2B]. In vitro matrix mineralisation was greatly increased in osteoblast pellets compared to monolayer culture [Fig.1C].

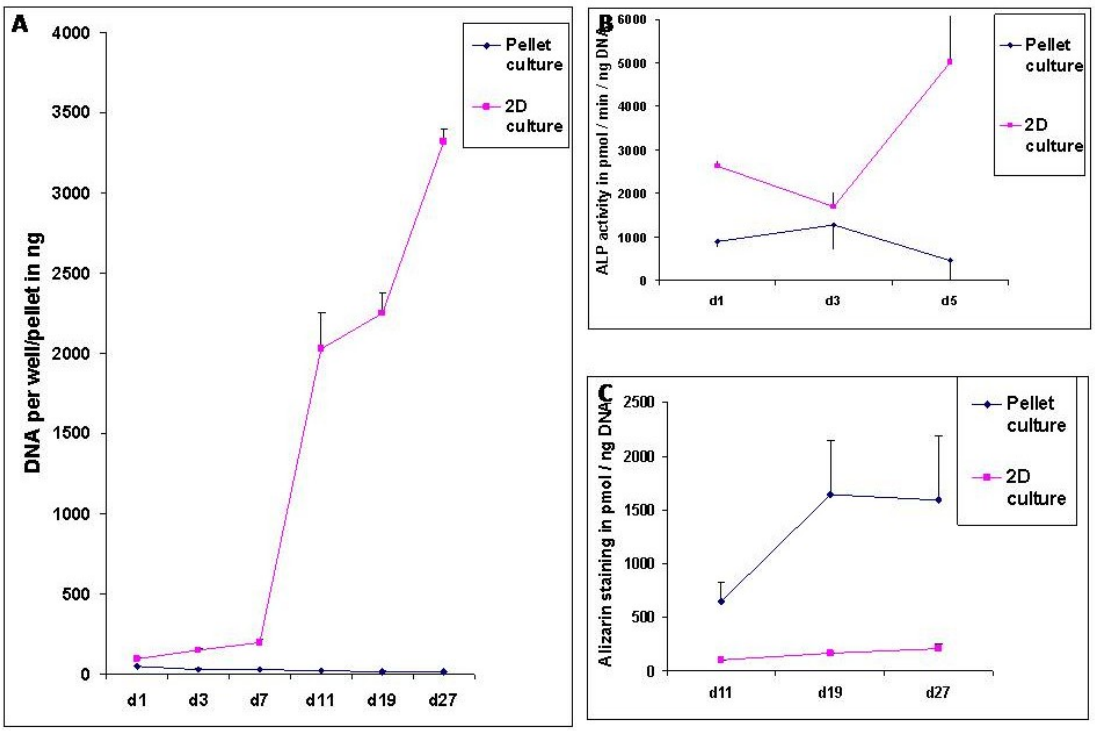

Fig.1: Monolayer and pellet culture of human osteoblasts.The graphs show the amount of DNA (A), ALP activity (B) and matrix calcium incorporation (C) over time.

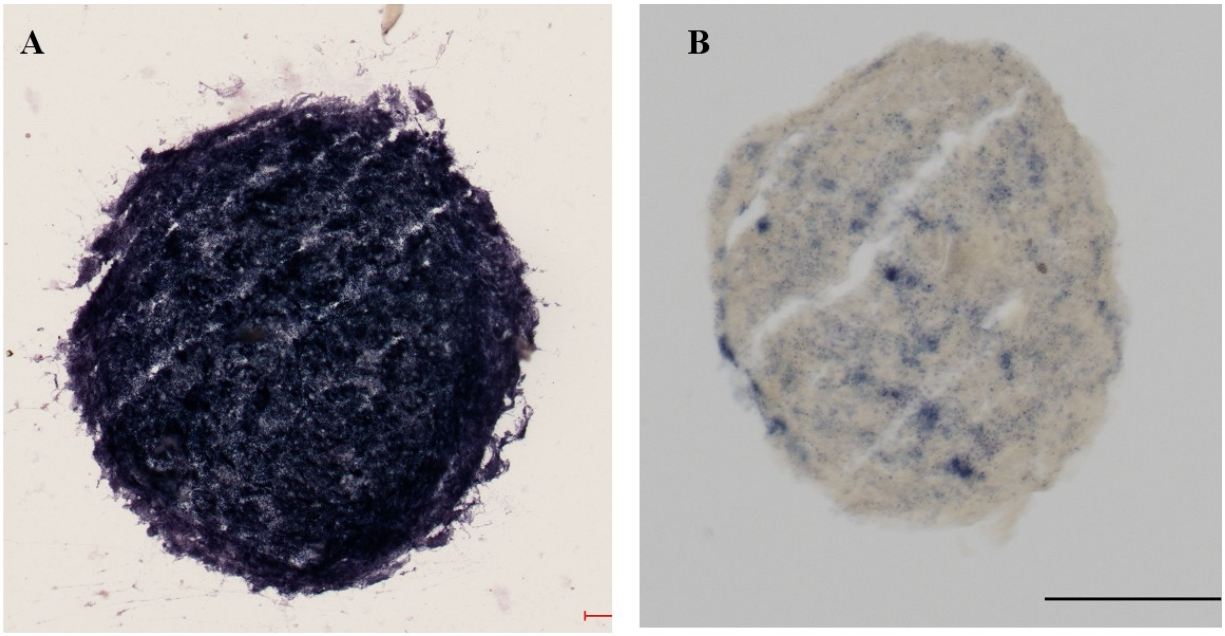

Fig.2: Micrographs of LDH (A; 7d) and ALP labelling (B; 11d) of cultured human osteoblast pellets. Scalebar represents 100μm.

DISCUSSION: A 3D culture model for primary human osteoblasts is introduced, which maintains overall cell viability up to 27d in culture. The exponential phase of cell proliferation as seen in 2D culture is absent. Interestingly, although ALP activity was greatest in monolayer culture, pellet culture demonstrated the highest matrix mineralisation. Morphological analysis of cultured cell pellets revealed at least two different cell populations – central cuboidal cells surrounded by flat surface lining cells.

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THE EVALUATION OF THE IN VIVO BONE FORMATION WITH THE EXPLORE OPTIX

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Background: When we try to approach the pathologic aggravation of metabolic bone diseases and a pharmaceutical effect, the evaluation of the bone metabolic turnover in vivo are extremely important. The conventional evaluation of bone formation is obtained by bone histomorphometry, which needs undecalcified bone specimen. We can get a couple of parameters of 'mineral appositional rate (MAR), bone formation rate (BFR) and osteoid suface (OS/BS)' etc related with bone formation by histomorphometry. However, this method requires much time for processing undecalcified bone specimen and histomorphometry. On the other hand, recently a fluorescent imaging device came to be used for biological analyses in vivo.

Objective: In the present study, we try to clarify the possibility of getting information about bone formation with fluorescence reagent 'calcein' injection to animals or not. Calcein is convenient reagent for bone labeling on calcification front.

Experiments: Ex.1: 6-week old and 10-week old of ICR female mice. Ex.2: OVX and Sham of ICR female mice. Ex.3: time course for normal ICR female mice. Evaluation of calcein uptake to 'Vertebra and coccyx' by the eXplore Optix was undertaken 24 hours later from calcein injection. The dose of calcein was 16mg/kg weight (s.c.). We have done all the procedure under isoflurane inspiration anesthesia. The measurement for calcein was as follows, emissionLaser (470nm), laser power (0 ~ 355 μ w), scan step (1.0mm), integration time (0.3sec).

Results: We could visualize calcein fluorescence signal taken in bone, using the eXplore Optix . We could clarify the differences between ages, and 'OVX vs Sham', with calcein injection, that is, the normalized photon intensity of 6-week old mice was two times higher than that of 10-week old mice (Ex.1), and the normalized photon intensity of OVX was 1.8 times higher than that of sham (Ex.2). Those results suggest that high-turnover bone was taken much more calcein. It is well known that those results were predicted with bone histomorphometry so far. In addition, we could get the data of the interval for one week (Ex.3).

Conclusion: The eXplore Optix could let us know the information of bone formation in vivo experiment without processing bone specimen.

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DRAGON, A GPI-ANCHORED MEMBRANE PROTEIN, INHIBITS BMP SIGNALING IN C2C12 MYOBLASTS

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Bone morphogenetic proteins (BMPs) induce osteoblastic differentiation of myoblasts through binding to cell surface receptors. Repulsive guidance molecules (RGMs) have been identified as BMP co-receptors. We report here that DRAGON/RGMb, a member of the RGM family, suppressed BMP signaling in C2C12 myoblasts via a novel mechanism. DRAGON and RGMa were expressed in both immature and mature C2C12 cells, but RGMc was detected only in mature cells. In C2C12 cells, only DRAGON suppressed ALP and Id1 promoter activities induced by BMP-4 or constitutively activated BMP type I receptors. This inhibition by DRAGON was dependent on the secretory form of the von Willbrand factor type D domain. DRAGON even suppressed BMP signaling induced by constitutively activated Smad1. Over-expression of neogenin did not change the inhibitory capacity of DRAGON. Taken together, these findings indicate that DRAGON may be an inhibitor of BMP signaling in C2C12 myoblasts. We also suggest that a novel molecule(s) expressed on the cell membrane may mediate the signal transduction of DRAGON to suppress BMP signaling in C2C12 myoblasts.

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SIMPLE STRATEGY FOR BONE REGENERATION WITH BMP-2/7 GENE EXPRESSION CASSETTE VECTOR

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Bone morphogenetic protein (BMP) is one of the most promising candidates for bone regeneration therapy. Heterodimers of BMP family proteins, such as BMP-2/-4 or BMP-2/-7, are well known to have stronger osteoinduction activity than BMP homodimers. Here, we constructed a double gene cassette vector encoding BMP-2 and BMP-7, pCAGGS-BMP-2/7, and examined its potential for osteoinduction in vitro and in vivo. The pCAGGS-BMP-2/7 vector had the equivalent ability to induce osteogenic differentiation in various cell lines as the combined transfer of expression vectors for BMP-2 and BMP-7. Moreover, the vector strongly induced bone formation in rat skeletal muscle when introduced by transcutaneous in vivo electroporation, compared with the introduction of BMP-2 alone. Thus, our BMP-2/7 double gene cassette vector, or some variation of it, may be applicable for the future clinical induction of bone formation, because it does not require multiple vectors or complicated preparation.

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COOPERATIVE INDUCTION OF CHONDROCYTE DIFFERENTIATION BY RUNX1 AND RUNX2

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Chondrocyte differentiation is strictly regulated by various transcription factors including Runx2 and Runx3. However, the physiological role of Runx1 in chondrocyte differentiation remains to be elucidated. To address that, we generated chondrocyte-specific Runx1-deficient mice (α1(II)Cre/Runx1flox mice) and mesenchymal cell-specific Runx1-

deficient mice (Prx1Cre/Runx1flox mice), because Runx1-/- mice die early in utero. Furthermore, we crossed them with Runx2 mutant mice to obtain chondrocyte-specific or mesenchymal cell-specific Runx1/Runx2 double mutant mice (α1(II)Cre/DKO mice and Prx1Cre/DKO mice, respectively).

α1(II)Cre/Runx1flox and α1(II)Cre/DKO mice were grossly normal. In contrast, Prx1Cre/Runx1flox mice displayed a delay in the calcification of the sternum, and Prx1Cre/DKO mice completely lacked the sternum. Notably, Runx1, Runx2 and Prx1 Cre transgene were co-expressed specifically in the sternum, explaining the restricted appearance of the abnormality. Histologically, in the prospective sternum of Prx1Cre/DKO mice, mesenchymal cell condensation developed normally, however, commitment to the chondrocytic lineage was seriously impaired. In line with that observation the expression of α1(□) collagen, Sox5 and Sox6 in the prospective sternum of Prx1Cre/DKO mice was severely attenuated by in situ hybridization, while Sox9 expression was unchanged. Molecularly, transient transfection of Runx1 in C3H101/2 cell increased Sox6 expression and it upregulated the promoter activity of Sox6. Moreover, in the promoter of Sox6, a potential Runx1 binding site was revealed by in silico analysis and, indeed, Runx1 bound to that site by EMSA. Accordingly, Runx1 was also recruited to this site by Chromatin Immunoprecipitation. Collectively, these results showed that Sox6 is the transcriptional target of Runx1.

In conclusion, we demonstrated that Runx1 and Runx2 regulate early chondrocyte differentiation through the induction of Sox5 and Sox6.

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LITHIUM CHLORIDE SUPPRESSES BMP4 ACCUMULATION FROM C3H10T1/2 CELLS TRANSFECTED WITH BMP4 EXPRESSION VECTOR

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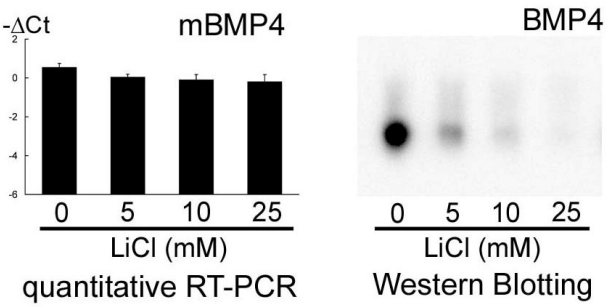
Lithium chloride is known as an inhibitor of glycogen synthase kinase-3 beta (GSK-3beta). Inhibition of GSK-3beta leads to accumulation of beta-catenin and activation of canonical Wnt signaling. The purpose of this study is to analyze the effect of canonical wnt signal on the expression of BMP4 and other related factors during chondrogenic differentiation of C3H10T1/2 induced by BMP4 gene transfer. C3H10T1/2 cells transfected with mouse BMP4 expressing plasmid by electroporation were used for experiments. A micromass culture with BMP4 transfected cells in medium containing ascorbic acid and ITS+ achieved chondrogenic differentiation. LiCl were added at 5,10 and 25 mM. The effect of LiCl was confirmed by luciferase activity of wnt signaling reporter construct, TOPflash. LiCl treatment increased the luciferase activity of TOPflash reporter assay in a does-dependent manner. Chondrogenesis evaluated by Alcian blue staining revealed marked decrease of chondrogenic property by LiCl treatment. The expression of BMP4 mRNA was not affected by LiCl. However, marked decrease of BMP4 protein by the addition of LiCl were seen in western blotting.(Figure) Also, immuno-staining for BMP4 on micromass showed decreased accumulation of BMP4 protein by LiCl. The anti-BMP4 antibody for protein analysis reacts with both pro-BMP4 and BMP4. Cathepsin H (ctsh) is previously reported to degrade BMP4 in the developmental stage of lung. The does-dependent upregulation of ctsh by LiCl was also seen in our RT-PCR examination. The knock-down experiment for ctsh showed significant increase of BMP4 protein accumulation by electro-transfection of siRNA for ctsh and BMP4 expression vector. These results suggest that Cathepsin H is regulated by canonical wnt signal and playing an important role in the post-translational regulation of BMP4.



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BONE MICRODAMAGE ACCUMULATES IN THE CENTRAL REGION OF THE HUMAN LUMBAR VERTEBRA

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The aetiology of back pain due to osteoporotic vertebral crush fracture, osteoarthritis, and/or ageing is poorly understood. Vertebral deformity, intervertebral disc disorganisation, and changes to vertebral bone architecture are morphological features that are associated with degeneration of the spine and with back pain. The aim of this study was to assess regional changes in cancellous bone microdamage present in the human lumbar vertebra. L2 lumbar vertebrae were obtained from 15 cadaveric spines (7 females, 8 males, mean age 62 [16-87] years). Sagittal slices cut from each vertebral body were *en bloc*-stained in basic fuchsin, and resin embedded. Each slice was cut into 9 sectors; for this study only the mid region (equidistant from the superior and inferior endplate) was analysed, specifically sectors (S)4 (anterior), S5 (central), and S6 (posterior). Morphometric assessment of bone architecture, resorption, and microdamage was undertaken. Trabecular bone volume (BV/TV), architectural parameters, and eroded bone surface (ES/BS) were not statistically different between sectors. However, S5 had the lowest BV/TV mean value compared to S4 and S6 (BV/TV[%]: S4:10.5 ± 5.8, S5:8.9 ± 4.1, S6:9.5 ± 4.6, p=ns), consistent with our published data (JBMR 16:681-687, 2001). Microdamage parameters were similar between S4 and S6. S5 had higher crack surface density (Cr.S.Dn) compared to S4 and S6 (p<0.03). Interestingly, crack density (Cr.Dn) and Cr.S.Dn associated negatively with ES/BS in S5 (r=-0.51, p<0.05; r=-0.57, p<0.03, respectively). Cr.Dn increased non-linearly with age for S4, S5, and S6. A negative association between Cr.Dn and BV/TV, approaching significance, was observed for S6 (r=-0.47, p=0.074), suggesting that changes in Cr.Dn are dependent upon the amount of trabecular bone in S6. Our data indicating reduced bone volume and increased microcrack burden in the central vertebral S5 compared with S4 and S6, provide further support for the central region of vertebrae being the weakest link in the structure of the human vertebral body. Further analysis of all 9 specified sectors will allow us to compare available biomechanical and intervertebral disc grading data with our microdamage data, to aid in determining how disc degeneration affects the mechanical properties of the bone and hence microdamage accumulation in the human lumbar spine.

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RBP4 IS DOWNREGULATED DURING SUTURE FUSION IN A MURINE MODEL OF SAETHRE-CHOTZEN SYNDROME.

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Craniosynostosis is a condition in young children involving the premature fusion of the fibrous suture tissue separating the calvarial bone fronts. The condition affects 1 in 2500 live births and can cause craniofacial abnormalities, aural and optical difficulties and mental retardation. Many environmental and genetic events have been implicated in the etiology of craniosynostosis, yet the molecular processes that cause this premature bone formation and suture fusion are unknown.

Following on from a recent microarray study within our laboratory which demonstrated a 37 fold downregulation of retinol binding protein 4 (RBP4) in fused versus unfused sutures of patients with craniosynostosis, our work aims to ascertain the involvement of retinol binding proteins during the process of suture fusion. The retinol binding proteins are a family of proteins involved in the traffic and storage of retinol and retinoic acid in the body. Until now they had not been recorded in suture tissue.

A cohort of twist heterozygous knockout (+/-) mice and their wildtype littermates were used. Analysis of suture fusion was performed via microCT scanning and confirmed using histological (H&E) staining. Gene expression analysis was executed using real time- quantitative PCR and analysed using the Delta-Ct method.

Coronal suture fusion, occuring as early as day 3 post partum, was present in twist +/- mice with the expression of RBP4 downregulated during the process of suture fusion. This downregulation of RBP4 appeared to directly correlate with coronal suture fusion in twist +/- mice. The osteoblast marker, osteocalcin was also analysed and seen to increase during bone formation.

The results of this study confirm the presence of retinol binding proteins in sutures, and their likely role in the maintenance of suture patency. Downregulation of RBP4 in fusing murine sutures mimics human sutures and confirms the usefulness of twist +/- as a mouse model of craniosynostosis.

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THE ROLE OF MYOD+ MUSCLE PROGENITOR CELLS IN BONE FORMATION AND REPAIR

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Introduction: It is well known that muscle cells can be forced to take on a bone-like phenotype in culture. However, their actual contribution to bone formation and repair was unclear. We aim to examine the capacity of myogenic progenitor cells to contribute to bone *in vivo*. Sophisticated model systems are required to track muscle cells once they have potentially adopted an osteoblastic gene profile.

Method: We utilised the MyoD-Cre transgenic line to conditionally label MyoD+ muscle progenitors. When crossed with the ROSA26R or Z/AP transgenic reporter lines, all MyoD+ cells and their descendents express the reporters LacZ or heat-resistant alkaline phosphatase (AP). We have examined the contribution of muscle-derived cells to bone in primary cell cultures, and in surgical models of heterotopic ossification (HO).

Results: Cell culture experiments using these transgenic mouse tissues confirmed that reporter genes are expressed in primary myoblasts, but not in other cell types. Moreover, reporter expression was maintained even after BMP-2 induced osteogenic differentiation. Histological staining established that LacZ and AP expression are limited to the skeletal muscles in the MyoD+/LacZ and MyoD+/AP lines respectively, with no contribution to normal bone development. MyoD+ cells were found to partially contribute to BMP-2 induced HO, and this input was increased when the muscle site was traumatised during surgery. Furthermore, the contribution of MyoD+ cells was greater at earlier time points, suggesting that these cells do not play a role in the persistence of heterotopic bone.

Conclusion: These data indicate that MyoD+ cells may not make as significant a contribution to HO as we initially hypothesised. Alternatively, high concentrations of exogenous BMP-2 may impair the onset of MyoD expression during satellite cell activation. We are currently addressing this issue by examining fracture healing where bone can form in the absence of high BMP levels. We are also probing new methodologies for examining the local vs. circulating cell contributions to HO, as well as the potential participation by other non-MyoD+ progenitor populations in muscle.

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MICRO-CT AND MICRO-ARRAY ANALYSES OF GROWTH PLATE CARTILAGE INJURY RESPONSES AND BONY REPAIR

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Growth plate cartilage is responsible for bone longitudinal growth. Injury to the growth plate is common and yet the injured cartilage is often repaired with undesirable bony tissue, leading to bone growth defects (limb length shortening and angulations). Using a rat model, our previous studies have shown sequential inflammatory, fibrogenic, osteogenic and bone maturation responses involved in the bony repair of the injured growth plate. However, little is known about the molecular mechanisms underlying the bony repair. In the current study, a drill-hole injury was inflicted at the proximal tibial growth plate of young rats, in vivo micro-CT was used to analyse time-course bone bridge formation, and microdisection, real-time RT-PCR, and microarray gene expression analysis were used to identify potential molecular mechanisms involved in bone bridge formation. Micro-CT analysis revealed that approximately 20% of the growth plate was disrupted by drill-hole injury. No bony material was detected within the injury site at day 1 and the amount of bone significantly increased at 14 and 60 days post-injury, where 8% and 30% of the injury site was replaced by bone, respectively. Interestingly, although there were no changes in growth plate thickness between injured

and normal rats at either day 14 or 60, at day 60, many small bone tethers formed at the adjacent growth plate outside the injury site but none was found in normal aged-matched rats. RT-PCR analysis with the adjacent uninjured growth plate (compared to normal age-matched controls) revealed differential expression of apoptosis-regulatory genes Bcl-2 and FasL and down-regulation of Sox-9 on days 7 and 14, whereas bone matrix protein osteocalcin was increased on day 60, suggesting degeneration and bone formation at the adjacent uninjured area. Microarray analysis of the injury site, collected using laser-capture microdisection, revealed differential expression of several components of the Wnt signalling pathway between days 4, 8, and 14 after the amplified cDNA was hybridized to rat Affymetrix gene chips. Other relevant pathways or processes identified include BMP signalling, chondrocyte, osteoblast, and osteoclast differentiation and skeletal development. Further analysis of microarray data will identify other molecular mechanisms and pathways involved in bone bridge formation.

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MATHEMATICAL MODELLING OF BONE REMODELLING IN FEMALE RATS

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The skeletal system undergoes continual replacement via remodelling, where osteoclast cells excavate resorption cavities, and osteoblast cells subsequently fill in the cavity by forming and depositing new bone material. To date, little is known about the cycle rate at which remodelling occurs, the size of resorption cavities, and the amount of formation deposited during a single remodelling event. Of particular interest is the modification of these features under conditions including skeletal disorders and administered treatments.

Here we model the basic relationship between trabecular structure and bone remodelling dynamics in terms of the three fundamental parameters: resorption, formation and cycle rate. Micro computed tomography data of trabecular bone from thirty rats collected over a period of twelve weeks were used to establish the changes in trabecular structure over time for three experimental groups: ovariectomy group; anti-resorptive treatment group; and a control group. A simple ordinary differential equation (ODE) was derived to describe the change in bone volume over time, and a model was developed to perform single remodelling events. This combined model was used to determine the three parameters of bone remodelling for separate experimental groups.

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THE ROLE OF MATRIX METALLOPROTEINASES (MMP'S) AND OSTEOCLASTS DURING ENDOCHONDRAL BONE GROWTH

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The process of endochondral ossification is essential to the achievement of skeletal growth. Osteoclast function has been associated with this process, however anti-osteoclastic agents such as Bisphosphonates (BP's) have failed to demonstrate strong negative effects. In contrast MMP activity has been suggested a key regulator of this process. We sought a clearer understanding of the role of MMP's and osteoclasts during endochondral bone growth.

In growing male rats, the following were administered subcutaneously for 2, 4 or 6 weeks. MMI270 (MMP inhibitor) twice daily at 120mg/kg, Osteoprotegerin (OPG) twice weekly at 10mg/kg, Clodronate (CLOD) at 30mg/kg twice weekly, Zoledronic Acid (ZA) at 0.05mg/kg twice weekly, or Saline.

Histological analysis of growth plates demonstrated increases of 30%-83% in growth plate height with MMI270 compared to Saline at each time point ($p<0.01$). These increases were due to an increased hypertrophic zone in these samples ($p<0.01$). Neither ZA nor CLOD treatment produced changes in growth plate height or morphology. In contrast, OPG treatment produced increases in growth plate height of 70% and 77% after 4 and 6 weeks of treatment respectively ($p<0.01$). OPG treatment also altered the morphology of the growth plate, with disorganization, limited zone distinction and irregular matrix mineralization. Osteoclast number at the chondro-osseous junction was reduced by 99-100% with OPG treatment compared to Saline after 2, 4 and 6 weeks of treatment ($p<0.01$). In contrast, MMI270 led to increases in osteoclast number up to 184% at 2 weeks and 47% at 4 weeks ($p<0.01$). CLOD also significantly increased osteoclasts here compared to Saline throughout the experiment, whereas ZA treatment produced a moderate increase in osteoclasts at 2 weeks only ($p<0.01$).

These results suggest that MMP activity may be crucial to the process of endochondral bone growth. Inhibition of osteoclast function with Bisphosphonates did not negatively impact the growth plate, suggesting a redundant function for these cells during this endochondral ossification. On the other hand, complete ablation of osteoclasts with OPG altered growth plate height and morphology . These results do not rule out a role of osteoclasts during this vital skeletal process. Further investigations are required into the possible direct effects of OPG on the growth plate.

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ENDOGENOUS G_I SIGNALING IN OSTEOBLASTS PARTICIPATES IN SEXUALLY DIMORPHIC REGULATION OF CANCELLOUS BONE STRUCTURE

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We have recently shown that expression of an engineered Gi coupled receptor under control of the Collagen I alpha 2.3 kb promoter results in severe trabecular osteopenia, yet little is known about the physiological role of endogenous Gi signaling in osteoblasts. In this study, we investigated the skeletal effects of blocking Gi signaling in osteoblasts *in vivo*. This was accomplished by transgenic expression in osteoblasts of pertussis toxin (PTX), which inhibits receptor-mediated activation of Gi signaling. Here we report that inhibition of endogenous Gi signaling in osteoblasts produces sexually dimorphic effects on cancellous bone. Female Col1(2.3):PTX transgenic mice showed significant changes in structural indexes of the trabecular network and a persistence of cancellous bone in the diaphysis, as assessed in femurs of 12wk old animals. This phenotype was described by microCT analysis of two consecutive regions of interest (ROI) beginning immediately below the primary spongiosa. Structural changes in the first ROI consisted of increased trabecular number (6.4±0.3 vs 8.7±0.5 per mm2, $p<0.01$) and connectivity density (430±50 vs 1050±100 per mm3, $p<0.001$), associated with reduced trabecular thickness (44.1±0.5 vs 36.6±0.7 μm, $p<0.001$). At this site there was no significant change in the fractional bone volume (BV/TV: 20.7±1.5 vs 23.7±1.8%). However, in the second ROI similar structural changes were associated with a marked increase (>65%) in BV/TV (9.8±1.3 vs 16.4±1.2%, $p<0.01$). Thus, in female mice, blockade of Gi signaling produced a marked increase in BV/TV specifically in the secondary spongiosa of the distal femoral metaphysis. In contrast male Col1(2.3):PTX mice displayed decreased BV/TV in both ROIs (first ROI:16.3±0.6 vs 11.1±0.9%, $p<0.001$; second ROI: 8.6±0.3 vs 7.0±0.4%, $p<0.01$). Initial histomorphometric results suggest that these dimorphic cancellous bone phenotypes were not associated with changes in either osteoclast or osteoblast surface for either gender. Increased osteoid width was noted in both genders, suggesting a role for endogenous Gi signaling in promoting normal mineralization. The persistence of cancellous bone in the diaphysis seen only in female Col1(2.3):PTX mice demonstrates that Gi signaling plays a complex role in the control of skeletal homeostasis that is dependent on both gender and the anatomic site within bone.

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PKCA REGULATES THE OSTEOBLASTIC DIFERENTIATION

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Purpose : Protein Kinase Cs (PKC) are involved in diverse physiological roles in many cells and identified to twelve isoforms based on their structure and cofactor regulation. Although the role of PKCα , one isoform of PKCs, has been

mostly reported in cardiovascular diseases, diabetes mellitus, and tumors, it has not been well elucidated in the osteoblastic differentiation. We herein report the role of PKCα in the osteoblstic differentiation.

Material and Method : We investigated the effects of two inhibitors of PKCs (Gö6976, PKCβ inhibitor) and one PKC stimulator (12-*O*-Tetradecanoylphorbol 13-acetate: TPA) on the osteoblastic differentiation in mouse preosteoblastic MC3T3-E1 cells. For the assay of the osteoblastic differentiation, we detected alkaline phosphatase (ALP) activity, gene expression of ALP, osteocalcin (OCN), type I collagen (Col I), and mineralization of extracellular matrix. In additon to these osteoblastic markers, transcriptional factors such as Runx2 and Osterix were assayed.

Result : Gö6976, which is PKCα and PKCβI inhibitor, stimulated ALP activity and mRNA expression of osteoblastic markers in dose-dependent manner. PKCβ inhibitor, which is PKCβI and PKCβII inhibitor, did not promote the osteoblastic differentiation in MC3T3-E1 cells. In contrast, TPA, which activated not only PKCα but other isoforms, suppressed osteoblastic differentiation in a dose-dependent fashion. Mineralizatoin of extracellular matrix was correlated with the result of ALP activity and mRNA expression. In the transcriptional factors, Runx2 was not influenced for PKCs inhibitors and activator.

Discussion : The osteoblastic differentiation was accelerated by Go6976, PKCα and PKCβI inhibitor, but not PKCβ inhibitor. Additionally, TPA, PKC stimulator, which also promoted PKCα activity, inhibited the differentiation. These results indicated that PKCα was involved in the osteoblastic differentiation and that its inhibition positively affected the differentiation.

Conclusion : PKCα might regulate the osteoblastic differentiation. The inhibition of its kinase could promote the differentiation. We are going to study signal transduction pathways of these phenomena.

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REGULATION OF OSTEOBLAST FUNCTION AND MINERALISATION BY EXTRACELLULAR NUCLEOTIDES: THE ROLE OF P2Y AND P2X RECEPTORS

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Extracellular nucleotides, signalling through P2 receptors, play a significant role in bone biology, modulating both osteoblast and osteoclast function. To date we have demonstrated $P2X_2$, $P2X_5$, $P2X_7$, $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptor expression by osteoblasts. We previously showed that ATP/UTP, potently inhibit alkaline phosphatase (ALP) activity and bone mineralisation *in vitro*, an effect which could be mediated, at least in part, via the $P2Y_2$ receptor. MicroCT analysis of 2-month-old $P2Y_2$ receptor deficient mice revealed increased femoral (43%, $p<0.01$) and tibial (21%) trabecular bone volume. Increases in femoral trabecular thickness (17%, $p<0.01$), trabecular number (33%, $p<0.05$) and cortical bone volume (25%, $p<0.01$) were also observed. Using qPCR and immunofluorescence we have now extended our investigation of P2 receptor expression by primary osteoblasts derived from rat calvariae. Osteoblasts were found also to express mRNA and protein for $P2X_1$, $P2X_3$, $P2X_4$, $P2X_6$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ receptors. Receptor expression changed with cellular differentiation; for example, $P2X_4$ receptor mRNA levels were 5-fold higher in mature bone-forming osteoblasts relative to immature, proliferating cells. To investigate whether receptors other than $P2Y_2$ might influence osteoblast function, osteoblasts were cultured for 14 days with the P2 agonists α,β-meATP, β,γ-meATP, Bz-ATP and 2-MeSATP (1-100μM). Mineralised bone nodule formation was measured by image analysis of alizarin red-stained cell layers. The $P2X_1$ and $P2X_3$ receptor agonists, α,β-meATP and β,γ-meATP (1μM) inhibited bone mineralisation by 70% and 90%, respectively, with complete abolition at $\geq$ 25μM. Bz-ATP, a potent $P2X_7$ receptor agonist, reduced bone mineralisation by 65% and 90% at 1μM and 100μM, respectively; this inhibition was abolished by the selective $P2X_7$ antagonist, A438079. The P2 agonist 2-MeSATP, which is not active at $P2Y_2$ receptors, also reduced bone mineralisation by >50% at 10μM. Osteoblast ALP activity was similarly decreased in each case by these agonists. These responses are consistent pharmacologically with involvement of the $P2X_1$, $P2X_3$, $P2X_5$ and/or $P2X_7$ receptors. These data highlight the expression of multiple P2 receptor subtypes by osteoblasts and indicate that extracellular nucleotides could function as local signaling agents that "switch off" bone mineralisation.

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AUTOLOGOUS BONE MARROW CELLS IMPLANTATION INDUCES BONE FORMATION IN A SHEEP SPINAL FUSION MODEL

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Bone marrow cells (BMCs), containing mesenchymal stem cells, have pluripotent potential to differentiate into multiple mesenchymal lineages, and presents as a potential cell source for bone tissue engineering, such as spinal fusion. The purpose of this study was to evaluate the efficiency of osteogenesis of sheep bone marrow cells in natural bone collagen scaffold (NBCS) in vitro, and the ability of these cells combined with NBCS to induced new bone formation in a sheep interbody lumbar fusion model. BMCs were co-cultured with NBCS for 1, 2, 3 and 4 weeks to investigate the proliferation, and differentiation of MSCs in NBCS by histological examination, scanning electron microscopy (SEM), immunohistochemistry and semi-quantitative RT-PCR. In sheep interbody fusion model, BMCs combined with NBCS were implanted in disc space, and was compared with autograft, NBCS alone and BMCs alone at 6 and 10 weeks postoperativily. In vivo results showed that sheep BMCs exhibited good proliferation 3-dimentionally in NBCS, which was evidenced by histology and SEM. Osteogenesis differentiation was induced in NBCS as well. Collagen type I matrix was also detected by immunohistochemistry, mineralization was demonstrated by von Kossa staining, and osteogenesis gene were investigated by RT-PCR. In vivo study showed that BMCs combined with NBCS yielded spinal fusion with higher fusion rate, greater biomechanical stiffness and larger bone volume than control groups. Histological finding also revealed that more mature new bone formation was induced by BMCs combined with NBCS, and integrated well with host bone tissue. We conclude that BMCs is a potential cell source for bone tissue engineering, and combined with NBCS, can be used as bone formation inducer for spinal fusion.

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INHIBITION OF BONE LOSS USING DYNAMIC MUSCLE STIMULATION IN A DISUSE OSTEOPENIA MODEL

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Musculoskeletal adaptations to aging and disuse environment have significant physiological effects on skeletal health, i.e., osteopenia. Mechanical loading through muscle stimulation (MS) has been shown to promote interstitial and blood flow to bone, possibly by creating a pressure gradient within the microvasculature and tissue[1]. The hypothesis for this study is that dynamic MS can enhance anabolic activity in bone, and mitigate bone loss in a functional disuse condition. Using a hindlimb suspension (HLS) rat model, dynamic MS was applied as replacement of the normal weight-bearing activity of the hindlimb with two skin patch electrodes at the right quadriceps muscles for total of 30 animals. The stimulus was applied at 115 mA, 71 Hz, 0.2 ms pulse with 3s on and 8s rest. The animals were divided into six groups (5 per group), including 1) baseline control, 2) age-matched control, 3) hindlimb suspended (HLS) sham control, 4) HLS+10 min MS, 5) HLS+30 min MS, 6) HLS+60 min MS, 5days per week, for a total of 4weeks. Left and right femurs were harvested for micro-computed tomography analysis. Distal metaphyseal regions and one epiphyseal region of the femurs (0.75mm per region) were scanned at 15 m m resolution and evaluated to obtain bone volume fraction (BV/TV), connectivity (Conn.D), and trabecular number (Tb.N). Disuse alone generated significant bone loss (-49.03% BV/TV, p=0.007, -58.55% Conn.D, -23.74% Tb.N, comparing to the age-match control). Dynamic muscle contraction at 30 min demonstrated anabolic effects at the metaphyseal regions (52.72% BV/TV, p=0.24, 76.38% Conn.D, 11.97% Tb.N, when comparing to the average HLS sham control). MS at 10 min showed a lesser response (7.31% BV/TV, 23.89% Conn.D, 3.17% Tb.N). The most significant response occurred at 60 min MS loading [103.43% BV/TV (p=0.003), 164.65% Conn.D, 22.35% Tb.N]. These results demonstrated that dynamic MS can initiate adaptive response to attenuate osteopenia under functional disuse environment with a dose dependent pattern, in which 60min low-energy contraction is able to recover 100% of bone loss induced by disuse. These data suggest that low-level bone strain and/or increased fluid pressure induced by MS may be crucial factors in musculoskeletal adaptation.

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PHOSPHODIESTERASE-MEDIATED ADAPTATION TO GS-ALPHA MUTATIONS IS DEVELOPMENTALLY REGULATED IN EMBRYONIC AND POST-NATAL STEM CELLS

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Fibrous dysplasia is a skeletal disorder caused by gain-of-function mutations of Gs-alpha. Although the genetic event occurs in early development, pre-natal skeletal growth is unaffected in FD patients. This suggests that skeletal progenitors display a different sensitivity to aberrant Gs signaling at different developmental ages. To address this issue, we infected mouse embryonic stem cells (ES) and human post-natal skeletal stem cells (Bone Marrow Stromal Cells, BMSCs) with a lentivector expressing the R201C rat Gs-alpha cDNA and analyzed their ability to differentiate into skeletal phenotypes. The expression of the transgene was confirmed at both RNA and protein levels. Surprisingly, neither ES cells nor BMSCs showed excess intracellular cAMP upon infection. However, treatment with IBMX revealed a different response in the two cell types. In transduced BMSCs, IBMX significantly increased intracellular cAMP compared to wild-type cells. Accordingly, high expression of IBMX-sensitive PDE isozymes (i.e. PDE 3, 4 and 7) was detected by q-PCR in basal conditions. IBMX alone did not modify cAMP levels in transgenic ES cells in which increased expression of the IBMX-insensitive PDE 8 was observed. Following incubation in specific inductive media, the expression of cartilage and bone markers was, overall, unaffected in mutated ES cells. Furthermore, mature cartilage undergoing endochondral ossification and normal bone were observed in teratomas generated upon in vivo transplantation. In contrast, differentiation of BMSC was profoundly affected by the mutation. In vitro, abnormal expression of skeletal markers was observed in specific differentiation assays. In vivo, ectopic osteogenic activity was limited to the deposition of a scarce amount of woven bone. In conclusion, these data demonstrate the different behavior of Gs-alpha mutated embryonic vs adult stem cells during skeletal differentiation. Most important, they show that a PDE-mediated adaptive response to Gs-alpha activating mutations operates in both cell types. However, different, and differentially regulated, PDEs isozymes are activated in mutated ES cells compared to postnatal skeletal progenitors, and the resulting adaptive response seems more efficient in ES cells than in postnatal stem cells. This could contribute to explain the normal development and abnormal postnatal growth of FD bone.

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THE ORPHAN NUCLEAR RECEPTOR, LIVER X RECEPTOR, INHIBITS BONE MINERALISATION

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The Liver X Receptor (α,β) is primarily responsible for regulating cholesterol homeostasis within cells and the whole body. However, as the role for this receptor is expanding we investigated whether the LXRs could be implicated in bone homeostasis. In studies with female LXRα-/-, LXRβ-/- and WT mice at 4 months of age, LXRβ-/- mice were found to have increased expression of Runx-2 in their long bones and a trend for osteoblast-associated genes (ie. osteocalcin (OC), osteopontin) to be elevated. Serum formation markers such as OC, alkaline phosphatase and leptin were also significantly increased. These results suggested that there was higher osteoblastic activity in the LXRβ-/- mice[3]. To elucidate the role for the LXRs in bone formation, we initially used the mouse osteogenic cell line MC3T3-E1 cells treated with the synthetic LXRα/β agonist GW3965, in the presence of differentiating media (50 mg/ml ascorbic acid, 10mM β-glycerophosphate, 50nM dexamethasone) for 21 days. We observed that the presence of GW3965, 24 hours prior to day 3, 5, 7, 14 and 21, significantly inhibited ALP and OC mRNA expression at these times. To see if longer treatment times would have a more significant effect, we treated with GW3965 for 3 days early in differentiation (days 5-8) and for 16 days (days 5-21). Both treatment intervals inhibited OC mRNA expression at day 21, the longer time inhibiting OC mRNA expression by 96% and protein by 64%. The MC3T3-E1 cells also formed significantly fewer bone nodules by day 21 shown by Alizarin red and Von Kossa staining. To further elucidate the mechanism we are currently treating MC3T3-E1 cells with GW3965 for the full 21 days and investigating osteoblast signaling pathways. Also, to study the apparent different roles of LXRα vs LXRβ, we are analysing the ability of bone marrow stromal cells derived from the LXR-/- mice to differentiate. Our study shows that LXRs, in particular LXRβ, have distinct roles in osteoblast function within the bone. The ability of the LXR agonist to inhibit mineralisation highlights a novel role for this nuclear receptor and expands the role for the LXRs.

[3] Robertson *et al* JBMR 21(8):2006.

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PROFILE ANALYSIS OF METAPHYSEAL TRABECULAR BONE IN RODENT OVARIECTOMY MODELS REVEALS A BIMODAL DOSE-DEPENDENT RESPONSE TO ADMINISTERED BONE ACTIVE AGENTS.

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At the knee metaphyses of long bones, in the rodent ovariectomy (OVX) model, there is a gradient of decreasing crossectional trabecular bone area with increasing distance from the growth plate. Here we re-analysed micro-CT image data from several OVX rodent studies to examine this "metaphyseal profile".

Four OVX studies were reviewed involving combined ovariectomy and drug treatment of young growing rats or mice aged (initially) 2-3 months. The 3D images of the whole metaphyses were analysed to assess the metaphyseal profile. These studies involved treatment with sex steroids, the bisphosphonate alendronate, PTH and the novel antiresorptive compound ABD295.

Measured metaphyseal profiles of crossectional trabecular bone area (BA/TA) were generally close to linear. Where metaphyseal trabecular bone volume in OVX rodents was restored by a specific drug treatment to levels similar to sham control levels, this gave a uniform elevation of the metaphyseal profile, but without change to its slope. Conversely, however, treatments which elevated trabecular volume to levels substantially greater than in sham controls ("high-responding" groups) were found to engender an upturn in the metaphyseal profile in the region closest to the growth plate. Drugs causing such high responses (at high doses) included sex steroids and alendronate.

These studies disclosed two distinct metaphyseal profiles that appear to represent a bimodal dose-dependent response to treatment. Partial or complete restoration of trabecular volume in OVX groups was shown to occur via a uniform alteration of the remodeling balance throughout the metaphysis. By contrast, in high-responding groups the upturned metaphyseal profile implicated an additional positive effect on the rate of trabecular bone formation near the growth plate.

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BMPR1A EXPRESSION CORRELATES WITH THE OSTEOGENIC SENSITIVITY OF MUSCLE PROGENITORS

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Osteoblasts are considered to primarily arise from osseous progenitors within the periosteum or bone marrow. Severe orthopaedic injuries can often result in substantial damage to these tissues, which can seriously impede bone repair. We have speculated that cells from local soft tissues may have the capacity to take on an osteogenic phenotype. Myogenic progenitors, vascular endothelial cells, and pericytes all represent potential sources of osteoprogenitors.

We have examined the osteogenic capacity of myoblasts, which are known to readily adopt a bone gene program upon treatment with the osteogenic bone morphogenetic proteins (BMPs) . We directly compared the BMP-2 sensitivity of myoblastic murine cell lines and primary cells with osteoprogenitors from osseous tissues and fibroblasts. BMP-2 induced a rapid and robust osteogenic response in myoblasts and osteoprogenitors, but not in fibroblasts. Outcome

measures included alkaline phosphatase expression, matrix mineralization, and the expression of osteogenic genes *(alkaline phosphatase, osteocalcin)* as measured by quantitative PCR .

BMPs signal via a receptors present on the cell surface. We hypothesized that the sensitivity of cells to BMP-2 would correlate with BMP receptor expression. We found that myoblasts and osteoprogenitors robustly expressed *Bmpr-1a*, and that *Bmpr-1a* levels increased with chronic BMP-2 treatment. Once cells began to express mature osteoblastic markers, *Bmpr-1a* began to decline, suggestive of a negative feedback mechanism. In contrast, fibroblasts expressed low levels of *Bmpr-1a* that was only weakly up-regulated by BMP-2 treatment.

We next speculated that we may be able to increase the osteogenic sensitivity of fibroblasts via forced myogenesis. Myogenic conversion was achieved by transduction with a MyoD-expressing lentiviral vector (LV-MyoD). Forced myogenic gene expression in fibroblasts was associated with a significant increase in the osteogenic response to BMP-2. Bioinformatics analysis identified the presence of myogenic responsive elements in the proximal promoter region of human and murine *BMPR-1A/Bmpr-1a*. Consistent with this prediction, *Bmpr-1a* expression was dramatically increased in LV-MyoD transduced fibroblasts.

These data demonstrate the osteogenic sensitivity of muscle progenitors and provide a mechanistic insight into the variable response of different cell lineages to BMP-2.

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WHOLE JOINT IMAGING ALLOWS QUANTIFICATION OF BONE AND CARTILAGE DEGENERATION IN A MURINE MODEL OF OSTEOARTHRITIS

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Osteoarthritis (OA) is a complex disease affecting both bone and cartilage and their subsequent breakdown appears to be interrelated. The literature for OA and its relationship to the subchondral bone is wide, varied and sometimes contradictory; however novel imaging-based research tools can provide versatile capabilities to investigate 3D architectural anomalies in both the bone and cartilage. In particular, the STR/ort spontaneous OA mouse model, used here, may be studied as disease progresses with age. In this work we employ 3D whole joint quantitative imaging techniques for assessment of subchondral bone and articular cartilage in this model, namely microcomputed tomography (μCT) and confocal laser scanning microscopy (CLSM), using the CBA/1 mouse strain as a control.

The whole knee joints of mice aged 3, 4, 7 and 10 months were scanned firstly with μCT at a 12 μm resolution, and then the tibial plateaus were scanned through the cartilage depth using CLSM. The results show that depending on site (medial and lateral), compartment (epiphyseal, metaphyseal, cortical), strain (CBA/1, STR/ort), and age (3, 4, 7, 10 months), the bone undergoes changes that lead to an altered architecture in arthritic knees. This is primarily seen as densification of the cortex and epiphysis in the medial condyles of the STR/ort mice, with a concomitant osteopenia in the lateral condyles (figure 1), alongside decreases in cartilage thickness and increased fibrillation (figure 2a). Furthermore, there is a significant change in many indices between 7 and 10 months. Specific cartilage surface is shown to correlate well with qualitative OA scoring ($R = 0.65$), where neither volume nor surface alone have the capacity to predict this damage (figure 2b).

Using a novel multimodal imaging approach, 3D morphometric changes in the murine osteoarthritic knee joint are elucidated. The results help explain relationships between changing bone architecture, cartilage breakdown and the progression of OA. Differences observed between the medial and lateral morphometry, in both the cartilage and bone indicate changes leading to altered mechanics in the OA joint. The STR/ort spontaneous OA model is shown to reflect human OA changes, emphasising its value for future studies, including investigation of targets for OA drugs and therapies.

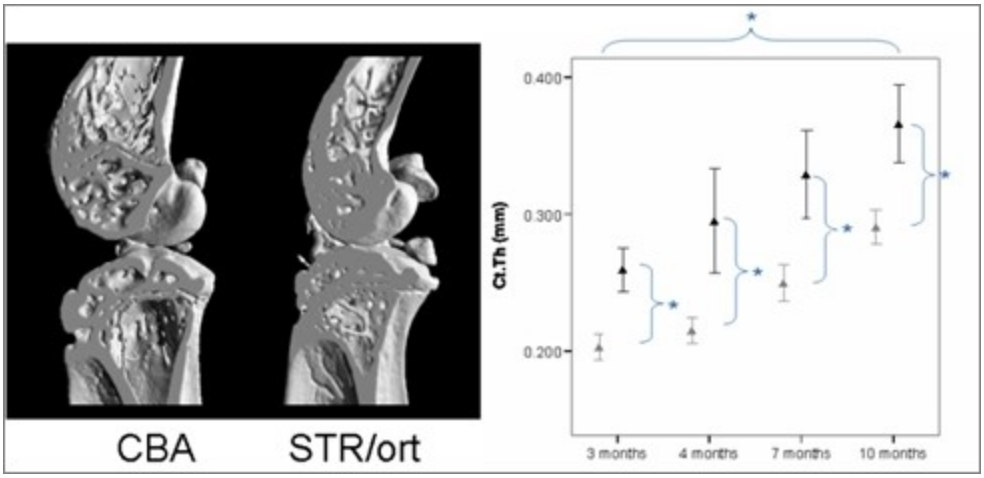

Figure 1: Bone changes at 10 months, and Ct.Th, grey: CBA, black: STR/ort, *p<0.05.

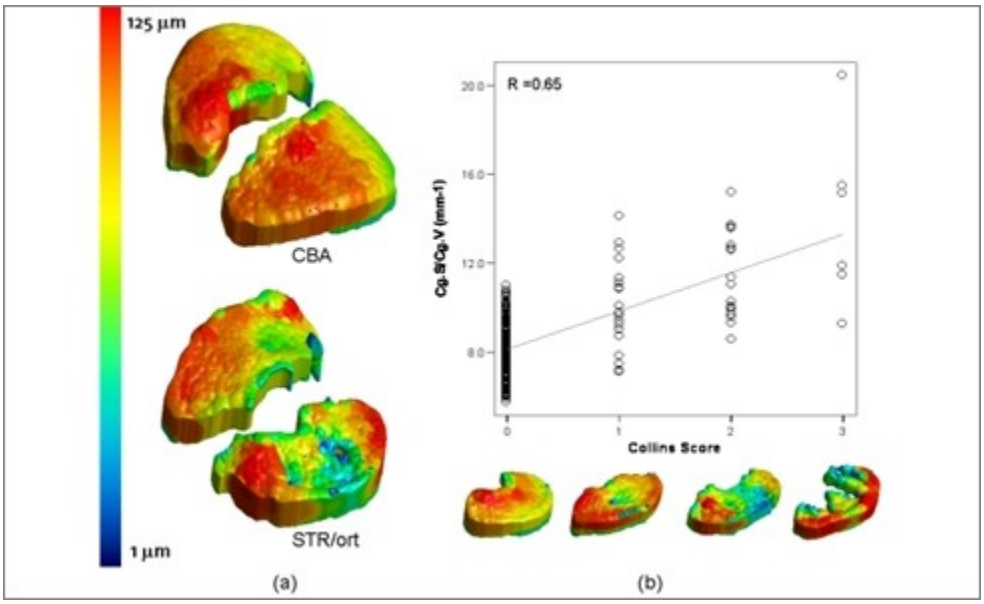

Figure 2: a) Comparison of cartilage with the onset of OA, and (b) correlation between qualitative and quantitative measures of degradation.

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EFFECT OF LOW-INTENSITY PULSED ULTRASOUND ON OSTEOBLASTS AND OSTEOCLASTS OF THE ZEBRAFISH SCALES

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Low-intensity pulsed ultrasound (LIPUS) provides noninvasive therapeutic treatment to accelerate fracture repair and distraction osteogenesis. However, most studies regarding the influence of LIPUS on bone metabolism have used osteoblast cell lines. An interaction between osteoclasts and osteoblasts has recently been noted in mammals, and it is necessary to consider the actions of both. Consequently, co-culture systems with both osteoclasts and osteoblasts are required for an accurate assessment of the effect of ultrasound stimuli on bone formation and resorption. The teleost scale is calcified tissue that contains osteoblasts and osteoclasts. Its bone matrix, which includes type I collagen and

hydroxyapatite, is similar to that of mammalian bone. In light of these findings, we recently developed an in vitro assay system with teleost scales as a marker, using alkaline phosphatase (ALP) for osteoblasts and tartrate-resistant acid phosphatase (TRAP) for osteoclasts. With this system, we examined the effect of LIPUS on the osteoblasts and osteoclasts of zebrafish scales. To analyze the detail mechanism of them, furthermore, mRNA expression in the scales treated by LIPUS was examined using a GeneChip system (Affymetrix).

Ultrasound was generated by the Sonic Accelerated Fracture Healing System (SAFHS, Exogen, Inc.) through a transducer (effective area: 3.88 cm^2) at a frequency of 1.5 MHz with a pulsed-wave mode (pulse-burst width; 0.2 s, pulse repetition frequency; 1 kHz, and intensity; 30 mW/cm^2). Scales were collected from zebrafish under anesthesia and then treated with LIPUS. After LIPUS treatment (20 min), the scales were incubated at 15 degrees C for 6 and 18 hrs with Leibovitz L-15 medium (Gibco). ALP and TRAP activities were then measured. ALP activity significantly increased by LIPUS at 6 hrs of incubation but did not change at 18 hrs of incubation. TRAP activity significantly suppressed at 6 and 18 hrs of incubation. By GeneChip analysis, the mRNA expression of osteoblastic markers such as periostin and type I collagen increased while the mRNA expression of osteoclastogenesis related factor (protein tyrosine kinase 2 B) decreased. Thus, we concluded that LIPUS promoted osteogenesis resulting from the influence of both osteoblasts and osteoclasts.

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CATECHOLAMINES ACCELERATE BMP-INDUCED OSTEOBLASTIC DIFFERENTIATION AND BONE FORMATION

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Introduction: It has recently been reported that the sympathetic nervous system is involved in regulation of osteoblastic function mainly through β-type adrenergic receptors on the cell surface. The stimulatory effects of the sympathetic nervous system are mediated by catecholamines, such as epinephrine, norepinephrine and dopamine, which elevate the intracellular cyclic adenosine 3', 5'-monophosphate (cAMP) level. In a previous study, we showed that intracellular cAMP accumulation consistently enhanced bone morphogenetic protein (BMP)-induced osteoblastic differentiation. This study was designed to substantiate the potential of these catecholamines to enhance BMP-induced bone formation.

Material and methods: To investigate the ability of catecholamines to augment the bone-inducing action of BMP under *in vivo* condition, 30 mg of polymer discs (poly-D, L-lactic acid - p-dioxanone - polyethylene glycol block copolymer; PLA-DX-PEG) containing rhBMP-2 (5 mg) with or without catecholamines (10, 20, 40 mg) were implanted into the dorsal muscle pouch of mice. All ossicles induced by BMP were harvested 3 weeks later and examined by radiological analyses. In murine bone marrow-derived stromal cells (ST2), changes of cAMP levels after addition of catecholamine were assayed in time sequence. The mRNA expression of Osterix, ALP and Osteocalcin (a marker enzyme of osteoblastic differentiation) and enzymatic activity of ALP were assayed by BMP treatment with or without epinephrine .

Results: *In vivo* condition, the ossicles induced by rhBMP-2 when used in conjunction with each catecholamine (epinephrine; 10, 20, 40 mg, norepinephrine; 40 mg, and dopamine; 10, 20, 40 mg) were significantly larger in size on soft X-ray radiogram and higher in bone mineral content on dual-energy X-ray absorptiometry, compared with those induced by rhBMP-2 alone. *In vitro* condition, intracellular cAMP levels were increased significantly within 2.5 minutes and maintained at least for 2h by cyclic addition of epinephrine. In the ST2 cells, the mRNA expression of Osterix, ALP and Osteocalcin and enzymatic activity of ALP were elevated significantly by BMP treatment (50ng/ml). These elevations were further elevated by addition of epinephrine (10^{-8}M).

Discussion and Conclusions: In this study, we showed that catecholamines accelerate BMP-induced osteoblastic differentiation and bone formation probably by increasing cAMP. In the body, catecholamine secretion and response may interact with BMP signaling, influence bone metabolism.

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OSTEOBLAST FUNCTION IS COMPROMISED AT SITES OF FOCAL BONE EROSION IN INFLAMMATORY ARTHRITIS

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In rheumatoid arthritis (RA), synovial inflammation results in focal erosion of articular bone. Despite treatment attenuating inflammation, repair of erosions with adequate formation of new bone is uncommon in RA, suggesting that bone formation may be compromised at these sites. Dynamic bone histomorphometry was used in a murine model of RA to determine the impact of inflammation on osteoblast function within eroded arthritic bone. Bone formation rates at bone surfaces adjacent to inflammation were similar to those observed in non-arthritic bone, therefore osteoblast activity is unlikely to compensate for the increased bone resorption at these sites. Within arthritic bone, the extent of actively mineralizing surface was reduced at bone surfaces adjacent to inflammation, compared to bone surfaces adjacent to normal marrow. Consistent with the reduction in mineralized bone formation, there was a notable paucity of cells expressing the mid to late stage osteoblast-lineage marker alkaline phosphatase, despite a clear presence of cells expressing the early osteoblast-lineage marker Runx2. In addition, expression of several members of the DKK and sFRP families of Wnt signaling antagonists was upregulated in arthritic synovial tissues. sFRP1 and sFRP2 mRNA expression was highest at days 7 and 8 post initial serum injection, corresponding to increasing inflammation and the onset of focal bone erosion within nearby bones. DKK1 mRNA expression peaked later in the time course of arthritis, with highest expression occurring around day 12 when inflammation and focal bone erosion is typically at its peak. DKK3 mRNA expression was elevated throughout the time course of arthritis. Interestingly, expression of mRNA for sFRP4, a WNT signaling antagonist that has been implicated in the inhibition of mineralization, was dramatically upregulated late in the time course of arthritis in tissues isolated from focal bone erosion sites. Together these data indicate that the presence of inflammation within arthritic bone impairs osteoblast capacity to form adequate mineralized bone at sites of focal bone erosion. Inhibition of Wnt signaling by multiple Wnt signaling antagonists, expressed by cells within the inflammatory tissues in RA, could be one mechanism contributing to impaired osteoblast function within arthritic bone.

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WNT/B-CATENIN SIGNALING PLAYS AN ESSENTIAL ROLE IN BONE SIALOPROTEIN-ELICITED OSTEOBLAST DIFFERENTIATION AND MINERALIZATION

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Bone sialoprotein (BSP) is one of the major non-collagenous phosphoproteins in bone and tooth. BSP stimulates differentiation of dura-derived cells into osteoblasts and subsequent osteogenesis in surgically created rat calvarial defects, but not in rat thoracic subcutaneous tissue, suggesting that BSP-elicited osteogenesis is dependent on local environment and/or the characteristics of responding cells [1]. This study was designed to examine the effects of cellular characteristics on BSP-mediated osteoblast differentiation and mineralization. To eliminate the effects of local environment on BSP action, primary cells isolated from rat dura or thoracic subcutaneous tissue were cultivated in the same conditions of α-MEM media. When the cells reached ~80% confluence, they were transfected with BSP expression lentiviral vectors with the addition of 10% FBS, 50 μg/ml ascorbic acid and 5 mM β-glycerophosphate. Transfection of rat dural cells with BSP expression vectors, but not empty vectors, led to the expression of osteocalcin (a marker of mature osteoblasts) and matrix mineralization at 14 days after transfection. However, forced BSP expression did not result in osteocalcin expression or mineralization in rat thoracic subcutaneous cells. Quantitative real-time PCR analyses demonstrated that expression levels of Wnt14, Wnt1 and its targets WISP1 and WISP2, and β-catenin were up-regulated in BSP-expressing dural cells, but not in BSP-expressing subcutaneous cells. To test whether canonical Wnt/β-catenin signaling plays an essential role in BSP-stimulated osteoblast differentiation and osteogenesis, β-catenin (the molecular node of canonical Wnt signaling) was stably knocked down in dural cells using β-catenin short interfering RNA lentiviral vectors prior to forced BSP expression. The results demonstrated that knock-down of β-catenin significantly suppressed the expression of osteoblast markers and the formation of mineralized bone-like nodules in cultivated BSP-expressing dural cells. Our data suggest that the initiation of BSP-elicited osteogenesis is

principally dependent on the intrinsic characteristics of responding cells, and that Wnt/β-catenin signaling plays an essential role in BSP-elicited osteoblast differentiation and matrix mineralization in dural cells.

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IS THERE A ROLE FOR BMPS IN SPINAL FUSION SURGERY?

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There has been a steady increase in the usage of BMP for spinal fusion surgery worldwide. However, the exact role of the various types of BMP, effective dose, carrier for BMPs and the cost-effectiveness is still unknown. The location, fusion technique and type of cage /allograft used are confounding factors in determining the real effects of BMP on spinal fusion.

Methods: A search of the literature from Medline and Embase until November 2008 was performed for clinical trials on the usage of BMP for spinal fusion. Inclusion criteria included all prospective cases using rhBMP-2 or rhBMP-7 for spinal fusion which utilized fine - slice computed tomographic (CT) scans to document fusion with a follow up of at least 2 years. The type of fusion procedure and the usage of allograft or type of cage had also to be defined.

Results: The studies included Anterior Interbody Fusion (5), Posterior Interbody fusion (2), Transforaminal Interbody Fusion (2), Posterolateral fusion (8) and 2 cervical discectomy and fusion. rBMP-2 was more effective than iliac crest bone in promoting posterolateral fusion ($P < 0.00001$), whereas rhBMP-7 (osteogenic protein-1) appeared equivalent to iliac crest bone ($P = 0.70$). There was a higher non-union rate in stand alone ALIF with BMP (44% vs 64%). The combination of rhBMP2 and allograft resulted in significant subsidence.rhBMP-2 usage in Transforaminal Interbody Fusion is associated with a higher incidence of heterotopic bone. Adverse events reported included ectopic bone formation, bone resorption or remodeling at the graft site, hematoma, painful seroma and neck swelling. Potential concerns with BMP usage include carcinogenicity and teratogenic effects.

Conclusions: There are varying rates of effectiveness of BMP with the various types of fusion techniques. In order to justify the routine usage of BMP, it has to be significantly more effective than the usage of autologous iliac crest bone graft while remaining cost-effective. Further studies would be needed looking into the suitable dosages for the various types of fusion techniques and the utility in high-risk fusion cases in osteoporotic patients and concurrent nicotine or NSAID use.

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CHARACTERISATION OF OVINE BONE MARROW DERIVED MESENCHYMAL STEM CELLS AND AUTOLOGOUS APPLICATION TO GROWTH PLATE CARTILAGE DEFECT

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Injury to growth plate cartilage can lead to bone bridge formation and bone growth deformities in children, a significant clinical problem currently lacking biological treatment. Although some small animal model studies highlight therapeutic potential of MSC for growth plate repair, translational research in large animal models, which more closely resemble the human condition are lacking. In addition, MSC from ovine bone marrow have been inadequately described. Previous studies have previously shown that ovine MSC share similar properties with human and rodents MSC, including their capacity for clonogenic growth and multiple stromal lineage differentiation. In the present study, ovine BM derived MSCs positively express surface markers associated with MSC including CD29, CD44 and CD166, and lacked expression of CD14, CD31, and CD45. Under serum-deprived conditions, proliferation of MSC occurred in response to EGF, PDGF, FGF-2, IGF-1, and most significantly TGF-α. While subcutaneous transplantation of ovine MSC with a ceramic HA/TCP carrier into immunocomprimised mice resulted in ectopic osteogenesis, adipogenesis and hematopoietic-support activity, transplantation of these cells within a gelatin sponge displayed partial chondrogenesis. Autologous MSC previously added to a gelatin sponge containing TGF-β1 were

transplanted into a surgically created defect of the proximal ovine tibial growth plate. While the transplanted MSC failed to form new cartilage structure at the defect site, the extent of osteogenesis was diminished and bone bridge formation was not accelerated due to the formation of a dense fibrous tissue. The comprehensive characterisation of ovine MSC described herein provides important information for future orthopaedic studies involving ovine MSC.

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IN VITRO STUDY OF DIFFERENT CHINESE HERBS IN CHONDROCYTE CULTURE: PROLIFERATION ASSAY AND COMP/ADAMTS-5 EXPRESSION

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Objective: To investigate the effect of different Chinese herbs on cell proliferation and Cartilage Oligomeric Matrix Protein (COMP) expression in chondrocyte culture. Methods: Chondrocytes enzymely isolated from rabbit knee cartilage were cultured within 3 generations with the density of 2×104 /cm2 and verified by collagen II immunohistochemical staining. Rabbit serums containing herbs were obtained after animals were administrated orally with dose equivalent to human body intake. At 5% and 10% serum density, cells were cultured in medium containing Liver-softening herbal compound serum. Subgroups setting of 1,3 and 5 hours after herb intervention were observed. Rabbit and bull serum were listed as control group.7days after intervention, chondrocyte proliferation was observed by using the MTT assay kit. For the study of COMP expression, chondrocytes were isolated from human knee cartilage. COMP level in supernatant was tested by enzyme-linked immunoabsorbent assays (ELISA) after directly adding compound and extract from Liver-softening herbs (HBP-A) to the culture with 10mg/ml density for 3 days. While being cultivated by HBP-A with 0.3mg/ml oncentration, ADAMTS-5mRNA expression was tested by ELISA and realtime PCR respectivwely.

Result: Liver-softening herbal compound group have significant effect on cell proliferation as compared to blank, of which,3 hour subgroup was better than 1 and 5 hour subgroup

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RAB3D DIRECTS ORGANELLE TRANSPORT BY RECRUITING CYTOPLASMIC DYNEIN MOTOR PROTEIN TCTEX-1 TO SECRETORY MEMBRANES OF OSTEOCLAST

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Transport of intracellular organelles along the microtubule cytoskeletal highway requires the coordinated activities of small regulatory GTPases, adaptor proteins and macromolecular motor complexes which act in a spatiotemporal manner to power directional motility. Rab3D, a non-neuronal member of the conserved Rab3 GTPase subfamily (Rab3A,-B,-C,-D), is a core component of the mammalian exocytic machinery . Like other G-proteins, Rab3D functions as a “molecular switch”, cycling “on” and “off” secretory granules and Golgi membranes in concert with its GTP-activation/GDP-inactivation states. This intrinsic cycling enables Rab3D to act at multiple facets of regulated

secretion through the recruitment of hitherto unknown GTP-dependent effector proteins. Here, we employed a yeast two-hybrid approach to identify Rab3D-binding partners. We identified Tctex-1, a light chain of the cytoplasmic dynein motor complex as a candidate Rab3D-microtubule coupling protein. Tctex-1 binds specifically to Rab3D and is recruited along with the dynein-dynactin complex to secretory granule and Golgi membranes following GTP-activation. In bone-resorbing osteoclasts, Rab3D-Tctex-1 co-occupies vesicle membranes and microtubules, an interplay which is disrupted upon depolymerisation of microtubule and disassembly of the dynein-dynactin complex. These data suggest that Rab3D directs microtubule-coupled organelle transport through the active recruitment of Tctex-1 and the dynein-dynactin complex to secretory membranes with potential widespread functional implications in secretion-competent cells.

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CALCIUM REGULATION IN FISHES: AN OVERVIEW

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Fish species come in great numbers (estimated 35.000, 60% of all vertebrate species) and inhabit a water continuum from soft, ion-poor water (e.g. Amazonian black water) to ion-rich seawater and concentrated salt water bodies (tidal rock pools). Freshwater and seawater fishes have calcium homeostasis independent of ambient calcium levels. Chloride cells (ionocytes) in the gills are key in calcium balance for continuous (skeletal) growth. Intestinal and renal secretion of calcium and phosphate secures the profile of divalent ions in the plasma. Calcium activities in water, cytoplasma, and blood plasma determine the permeabilities of membranes and epithelial junctional complexes, control ion channel activities and plasma membrane electrical properties. The epithelial electrophysiology and transporter make-up of the plasma membrane, dependent on ambient calcium levels and endocrine responses to the ambient calcium, determine net movements of calcium. Flux to internal stores (bone and scales) further sets the balance for calcium. Fishes may continue to grow throughout their life, and their bone represents an important calcium and phosphate buffer compartment. Fishes 'evolved' bone as we find it in vertebrates; gill calcium transport may serve a major role to supply the growing bone with mineral. Calcium sensing in and outside the cell, direct and indirect regulation of calcium activity, calcemic controls will be reviewed with a focus on recent insights in the endocrinology of prolactin, parathyroid hormone related protein and parathyroid hormone, calcitriol , calcitonin, stanniocalcin and the enigmatic beta-glucuronidase klotho.

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N-3 AND N-6 LONG-CHAIN POLYUNSATURATED FATTY ACIDS DIFFERENTIALLY MODULATE IL-6 SECRETION IN LIPOPOLYSACCHARIDE-STIMULATED MURINE OSTEOBLASTS

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Pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α are known to be active in the pathogenesis of osteoporosis. Results from clinical trials and *in vivo* animal studies suggest that specific long chain polyunsaturated fatty acids (LC-PUFAs) especially those of the n-3 PUFA family, might be beneficial for bone health. In order to elucidate possible cellular mechanisms, the effects of some LC-PUFAs, representative of the n-3 and n-6 families, were investigated on osteoblastic secretion of various inflammatory cytokines.

Lipopolysaccharide-stimulated murine MC3T3-E1 osteoblasts were exposed to ethanol (vehicle control), arachidonic acid (AA), gamma-linolenic acid (GLA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 20 μg/ml for periods of 2.5h to 20h. Conditioned media were collected and cytokine levels determined with a Mouse Th1/Th2 10plex FlowCytomix Multiplex kit (BenderMed Systems) and expressed in pg/ml. Three independent experiments were conducted (n=4).

Negligible levels of the pro-inflammatory cytokines IL1-α and TNF-α were detected in the conditioned media of all samples tested. Compared to the respective controls, AA (n-6) stimulated IL-6 secretion significantly by 140-200%, which was evident after 2.5h already. GLA (n-6) and EPA (n-3) enhanced IL-6 secretion by 10-80%, reaching a maximum after 10-15h of exposure. DHA (n-3) had a pronounced inhibitory effect on IL-6 levels.

Prostaglandin E_2 (PGE_2) has been shown to stimulate IL-6 secretion. The stimulatory effect of AA on IL-6 secretion could be attributed to the production of PGE_2 that is derived from AA. In our study, this is confirmed by the

observation that co-incubation of AA with the cyclo-oxygenase-2 blocker NS-398 (20 μM) significantly attenuated stimulation of IL-6 secretion. Enhancement of IL-6 secretion by GLA and EPA may be associated with production of PGE_1 and PGE_3 respectively. No prostanoids are derived from DHA, suggesting that the inhibitory effect of this n-3 LC-PUFA on IL-6 secretion could act through a different pathway. Further work is needed to understand the modulation of various cytokines by the LC-PUFAs in bone cells.

This work was supported by the National Research Foundation (South Africa).

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HUMAN FETAL OSTEOBLASTIC 1.19 CELL LINE AS AN IN VITRO HUMAN MODEL TO STUDY THE MECHANISM OF PARATHYROID HORMONE ANABOLIC ACTION ON BONE

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Parathyroid Hormone (PTH) is well known to have a catabolic action on bone. It releases calcium from bone through the activation of bone resorption by osteoclasts. However, when given intermittently, PTH has a paradoxical anabolic action on bone resulting in enhanced bone formation and mineralization. It has been proposed that PTH increases osteoblasts numbers through increased osteoblastogenesis, inhibition of apoptosis, or activation of the lining cells to become osteoblasts. Up to date, the exact mechanism of the anabolic action of PTH on bone is not fully understood. Moreover, and to the best of our knowledge, previous in vitro studies merely relied on animal cell lines which could be far from replicating the physiology of human cells.

The human fetal osteoblastic 1.19 cell line (hFOB 1.19) is a well-studied and commercially available clonal human osteoprogenitor cell line with multilineage differentiation capability. They are in early differentiation stage and can easily be used to study the effects of PTH on the proliferation and differentiation of human osteoblasts.

We examined the effect of human PTH (1-84) on the proliferation of hFOB 1.19 cells. Cells were incubated under 2.5 nM, 25nM, and 250 nM PTH for 4, 8, and 24 hours in a serum free medium. The proliferation was measured using a BrdU proliferation assay on day 5. The experiments were performed in quadruplets.

Treatment with PTH enhanced the proliferation of hFOB 1.19 in a dose and time dependent manner. The highest proliferation effect was achieved when cells were incubated under 250 nM PTH for 4 hours. These results demonstrate that transient PTH stimulates osteoblasts proliferation in vitro which could in part explain the mechanism of the anabolic action of PTH in bone. They also endorse the potential of hFOB 1.19 cells to be used in such investigations in the future.

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C-MYB EXPRESSION AT DIFFERENT STAGES OF MOLAR TOOTH GERM OSSEOINTEGRATION

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The c-Myb protein is a transcriptional factor that plays a role in regulation of cell proliferation, differentiation and apoptosis. The c-myb gene is constitutively expressed in hematopoietic cells and other rapidly dividing cells as e.g. in the colon and tumour tissues. Due to this fact, c-myb is particularly investigated in oncological studies. However, c-myb expression was detected also during embryonic development and seems to be involved in the development of tooth germs in context of surrounding bone tissue.

Odontogenesis is based on reciprocal epithelio-mesenchymal interactions and the first sign is a molecular cross-talk. Starting embryonic day E12.5 the first mouse molar tooth bud becomes apparent morphologically and gradually progresses to the cap and bell stages creating form for the mineralized tooth. Simultaneously, the surrounding structures of future interdental bone develop and mineralize. At the point of the second and third molar tooth germ development, the bone appears fully ossified and the growing tooth must establish the required space.

Therefore, the aim was to show c-myb expression profile in temporo-spatial model of mouse molar tooth germ development in the context of related tissues. Expression of c-myb (Abcam polyclonal antibody) was followed together with proliferation analysis (PCNA –proliferating cell nuclear antigen, DAKO monoclonal antibody) and apoptosis

detection (TUNEL test, terminal deoxynucleotidyl transferase mediated dUTP nick end labelling, Chemicon). Secondary biotinylated antibody was labelled by streptavidin-POD to visualize positive cells by addition of DAB substrate. c-myb, PCNA and TUNEL were evaluated in the epithelial and mesenchymal parts of the tooth germ and in the surrounding interdental bone.

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Category 7. Cancer and Bone

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SOLUBLE RANK LIGAND PRODUCED BY MYELOMA CELLS CONTRIBUTES TO GENERALISED BONE LOSS IN MULTIPLE MYELOMA.

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Patients with multiple myeloma commonly develop focal osteolytic bone disease, as well as generalised osteoporosis. The mechanisms underlying the development of osteoporosis in patients with myeloma are poorly understood. Although disruption of the RANKL/OPG pathway has been shown to underlie formation of focal osteolytic lesions, its role in the development of osteoporosis in myeloma remains unclear. Increased soluble RANKL in serum from patients with myeloma raises the possibility that this molecule plays a key role. This study aimed to establish whether sRANKL produced by myeloma cells contributes directly to osteoporosis in experimental models of myeloma.

C57BL/KaLwRij mice were injected with either 5T2MM or 5T33MM murine myeloma cells. 5T2MM-bearing mice developed osteolytic bone lesions ($p<0.05$), with increased osteoclast surface ($p<0.01$), and reduced trabecular bone volume ($p<0.05$). Bone volume was also reduced at sites without histologically detectable 5T2MM cells ($p<0.05$). Soluble mRANKL was increased ($p<0.05$), whereas OPG was not altered, in serum from 5T2MM-bearing mice. In contrast, 5T33MM-bearing mice had no changes in osteoclast surface or trabecular bone volume and did not develop osteolytic lesions. Soluble mRANKL was undetectable in serum from 5T33MM-bearing mice. In separate experiments, RPMI-8226 human myeloma cells were stably transfected with human RANKL/eGFP, or eGFP alone. RPMI-8226/hRANKL/eGFP cells, but not RPMI-8226/eGFP cells, stimulated osteoclastic bone resorption ($p<0.05$) *in vitro*. Sub-cutaneous injection of NOD/SCID mice with RPMI-8226/hRANKL/eGFP or RPMI-8226/eGFP cells resulted in tumour development in all mice. RPMI-8226/hRANKL/eGFP-bearing mice exhibited increased serum soluble hRANKL ($p<0.05$) and a three-fold increase in osteoclast number ($p<0.05$) compared to RPMI-8226/eGFP-bearing mice. This was associated with reduction in trabecular bone volume (27%, $p<0.05$), decrease in trabecular number (29%, $p<0.05$) and increase in trabecular thickness (8%, $p<0.05$).

Our findings demonstrate that soluble RANKL produced by myeloma cells contributes to the generalised osteoporosis and suggest that targeting RANKL may prevent osteoporosis in patients with myeloma.

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PREDICTION OF PATHOLOGICAL FRACTURE OF THE FEMORAL SHAFT USING CT IMAGE BASED 3-DIMENSIONAL FINITE ELEMENT METHOD

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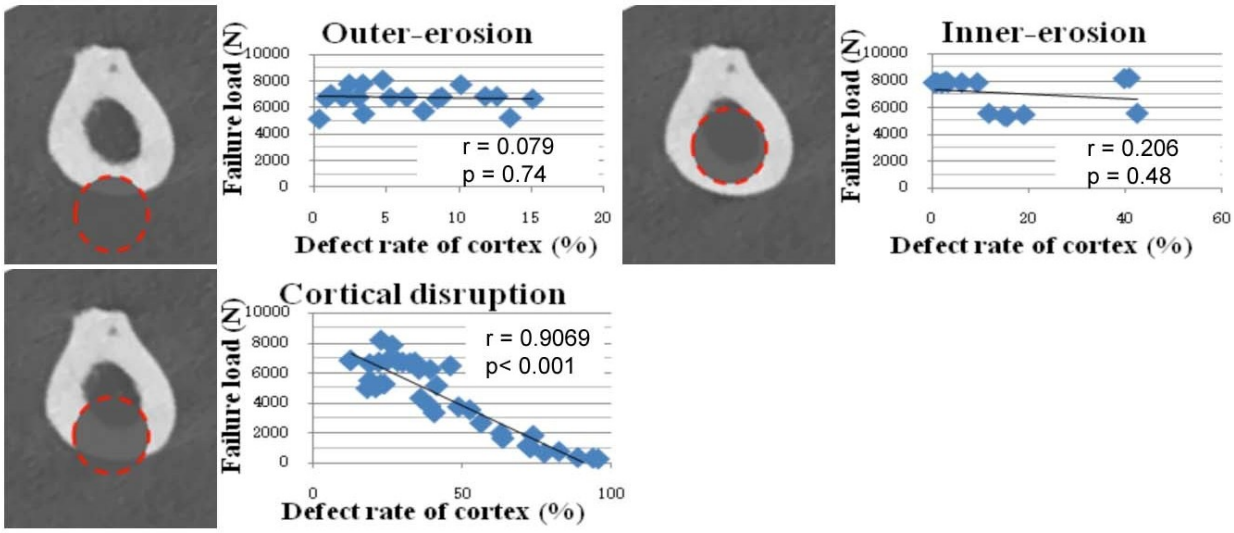
PURPOSE: To clarify the relationship between the presence of osteolytic lesion due to metastatic bone tumor in the femoral shaft and the reduction of its mechanical strength using three dimensional finite element (FE) analysis.

MATERIAL and METHODS: FE models were developed from the computed tomographic data using software, Mechanical Finder. Both the elastic modulus and the strength in each element were calculated based on the data reported by Keyak. On the other hand, Poisson's ratio was determined using the data reported by Minamisawa. A

spherical defect was created at the level of the isthmus in the femoral shaft to simulate the presence of osteolytic lesion. The diameters of the defect were determined as 5, 10, 15, 20, 25 and 30 mm. Each defect was moved 2 mm stepwise to the ventral direction on the horizontal plane. Boundary conditions were determined to represent load configurations, approximating joint loading during single limb stance. The relationships between the failure load and the cross-sectional area of the cortical bone loss or the types of osteolytic lesions were analyzed. The shape of the bony defect was classified into three types. The inner-erosion was defined as an osteolytic lesion localized inside the bone. The cortical disruption was the type in which a defect perforated through the entire cortex. The outer-erosion was defined as an osteolytic lesion seen only in the outer surface of the cortex.

RESULTS: The correlation between the failure load and the defect rate of the cortex was not statistically significant either in the inner-erosion or in the outer-erosion type. On the other hand, there was a strong negative correlation between the failure load and the defect rate of the cortex in the cortical disruption type.

DISCUSSION AND CONCLUSION: Our results clearly demonstrated that the strength of the femoral bone correlated negatively with the extent of the cortical defect in the femur with cortical disruption. These results could be utilized in the clinical practice for the risk assessment of the pathological fractures due to osteolytic metastatic bone tumors.



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DAMAGE AND RECOVERY OF BONE MARROW MICROENVIRONMENT AFTER ACUTE METHOTREXATE CHEMOTHERAPY

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Disruptions to interactions between cells of stromal and haematopoietic lineages caused by chemotherapy treatment alter steady-state proliferation, differentiation and maintenance of stem cell niches, and are associated with skeletal defects known to develop after intensive chemotherapy treatment. The anti-metabolite dihydrofolate reductase inhibitor Methotrexate (MTX) is used commonly to treat Acute Lymphoblastic Leukemia and some solid tumours, known to cause haematopoietic toxicity and bone loss in cancer patients. However, the cellular/molecular mechanisms associated with MTX damage to the bone and bone marrow are yet to be fully elucidated. In this study, short-term MTX chemotherapy (five once-daily injections at 0.75mg/kg/day) was conducted in rats to investigate the damage to and subsequent recovery of the bone marrow microenvironment and the potential role of the CXCL12/CXCR4 axis. Marrow cell density 6, 9 and 10 days after the initial MTX dose was significantly reduced, which was accompanied by an increase in marrow adipocyte number, although both returned to normal by day 14. Consistent with the reduced cellularity, MTX caused a reduction in marrow cell proliferation on days 6 and 9 as measured by BrdU labelling. In addition, a CFU-GM assay of isolated marrow cells was used to estimate contents of committed granulocyte-macrophage progenitor cells, which revealed a reduction in colonies on day 6 but recovery on day 9. Consistent with the increase in adipocyte number, there was an increase in adipocyte formation in an *ex vivo* assay of isolated bone marrow stromal cells and an increase in adipogenic differentiation regulator PPAR-g expression in bone. To investigate the effects of MTX on marrow stromal progenitor numbers, a CFU-F assay plus Alkaline Phosphatase staining was

performed, which revealed a significant decrease in positive colonies on days 6 and 9 returning to near normal by day 14. This study illustrates that acute MTX chemotherapy transiently depletes the bone marrow of its steady-state haematopoietic and stromal progenitors, reducing haematopoietic cellularity and osteogenesis and increasing marrow fat content, which have the capacity to recover by day 14 subsequent to damage.

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ASSESSMENT OF VITAMIN D STATUS AND DEFINITION OF A NORMAL CIRCULATING RANGE OF 25-HYDROXYVITAMIN D

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Purpose: The assessment of circulating 25-hydroxyvitamin D [25(OH)D] for clinical diagnosis has increased in an exponential fashion during the past 5 years. It is thus timely to review the reasons for this increase as well as the diverse analytical methods used to meet this need.

Recent findings: Nutritional vitamin D status, as defined by circulating levels of 25(OH)D, has long been implicated in skeletal health. However, in the past decade circulating 25(OH)D has been strongly linked in humans to cancer rates, autoimmune disease, cardiovascular health and infectious disease. As a result, availability and rapid analytical turnaround of 25(OH)D assays have had to improve. Today these demands are largely met in the clinical laboratory by direct automated chemiluminescent platform analysis or highthroughput LC/MS procedures. These methods are diverse and often do not agree with respect to designated reference ranges.

Summary: The assessment of circulating 25(OH)D levels has become an important clinical tool in the management and prevention of diverse disease states. For this reason, assay standardization as well as a uniform reference range for circulating 25(OH)D levels must be achieved.

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ZOLEDRONIC ACID INHIBITS BOTH THE OSTEOLYTIC AND OSTEOBLASTIC COMPONENTS OF OSTEOSARCOMA LESIONS IN BOTH AN IMMUNODEFICIENT AND IMMUNOCOMPETENT ANIMAL MODEL.

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Purpose: To evaluate and compare the efficacy of Zoledronic acid (ZOL) against osteosarcoma (OS) growth, progression and metastatic spread using an immunodeficient mouse model of human OS and an immunocompetent rat model of OS.

Experimental Design: Human K-HOS OS cells, tagged with a luciferase reporter construct were transplanted directly into the tibial cavity of nude mice. In a separate experiment, rat MSK-8G OS cells were transplanted into the tibial cavity of Fischer 344 rats. ZOL was given as either a weekly, or single dose of 100 μg/kg body weight, equivalent to the 4-mg intravenous dose given to patients. Tumour growth at the primary site and as pulmonary metastases was monitored by bioluminescence imaging or histology and OS-induced bone destruction was measured using high resolution micro-CT.

Results: Both mice (n=10 per group) and rats (n=6) transplanted with OS cells exhibited aberrant bone remodeling in the area of cancer cell transplantation, with areas of osteolysis mixed with an extensive network of new bone formation extending from the cortex. ZOL administration prevented the formation of osteolysis and significantly reduced the

amount of OS-induced bone formation. However, ZOL had no effect on tumor burden at the primary site in either model. Importantly, we found that treatment with ZOL resulted in significantly increased pulmonary metastases when compared to vehicle in the immunodeficient mice only but not the immunocompetent rats.

Conclusions: ZOL inhibits the development and progression, not only of the osteolytic, but also the osteoblastic component, of OS lesions in animal models that closely resemble the human disease. T he clinical relevance of the observed increase in pulmonary metastases induced by ZOL treatment in the immunodeficient mouse model but not the immunocompetent model, suggests the involvement of the immune system, and warrants further investigation .

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LOCALISING INDIVIDUAL MYELOMA CELLS TO THE MYELOMA 'NICHE' IN BONE USING MULTIPHOTON MICROSCOPY.

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The myeloma ‘initiating' cell is likely to reside in a specialised ‘niche' in bone, however defining this environment has proved challenging. In experimental models of myeloma, inhibiting bone resorption or promoting osteoblastogenesis reduces tumour burden, suggesting that bone cells may contribute to the myeloma ‘niche'. Haemopoietic stem cells, also reside in a specialised endosteal osteoblast ‘niche'. Whether myeloma cells occupy the same, or a closely related, ‘niche' is unknown. The aim of this study was to determine whether individual colonising myeloma cells localise to a specific microenvironment in bone.

5T33MM murine myeloma cells were stably transduced with eGFP (5T33MMeGFP). C57BL/KaLwRijHsd mice were injected, intravenously, with 5T33MMeGFP cells, 5T33MM cells transduced with a control vector (eGFP negative), or PBS. Mice were sacrificed after 18 hours, the tibiae frozen and the bone marrow space exposed by sectioning on a cryostat. The whole tibia was analysed on a Zeiss 510 META multiphoton microscope. Images were then reconstructed and analysed using Volocity software (Improvision). Objects smaller than 25 μm^2 and larger than 900 μm^2, which represent cells contained in blood vessels were excluded. Analysis of 2D ‘tiled' reconstructed sections of the whole tibia revealed few if any eGFP fluorescence in mice injected with PBS or 5T33MM cells bearing the control vector. In contrast, the tibia of mice bearing 5T33MMeGFP cells contained individual tumour cells distributed throughout the metaphysis. Analysis of 3D ‘tiled' z-stacks revealed individual 5T33MMeGFP cells directly adjacent to bone surfaces. A limited number of cells were found in the bone marrow space. These data demonstrate that mulitphoton microscopy can be used to visualize individual myeloma cells in bone and they locate to bone surfaces. Mapping the precise cellular localize should enable us to determine the nature of the myeloma ‘niche' and lead to novel approaches to preventing the development of myeloma.

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THE VISCOELASTIC RESPONSE OF TUMOR-METASTASIZED BONE USING DYNAMIC MECHANICAL ANALYZER

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Introduction: Bone metastasis is prevalent in many malignancies, such as breast, prostate, thyroid, renal and lung cancers. The most common site for bone metastasis is the proximal extremity of long bones. In patients with breast cancer, cancer cells which successfully metastasize to bone promote osteolytic and cause destructive bone lesions. To quantify this change, Bone Mineral Density (BMD) is typically used to measure bone quality and for diagnoses to be made. However, BMD values alone cannot predict fracture risk in metastasized-bone; it is possible for bones with larger BMD values to have higher risk of fracture than those with smaller BMD values. Besides BMD, viscoelasticity is another parameter that can potentially measure bone quality. The purpose of this study is to investigate the difference in viscoelasticity between tumor-implanted and sham-operated bones.

Methods: W256 malignant breast cancer cells were injected directly into the left distal femur of female Sprague Dawley rats, while a sham operation was performed on the other femur. The animals were euthanized and both femurs were harvested after 30 days. Viscoelasticity was obtained using the Dynamic Mechanical Analyzer (DMA), which produces an oscillating stress on the bone specimen so that the resulting strain can be measured. Specimens were

subjected to oscillatory bending loading in a single cantilever (frequency range : 0.001Hz ~ 70Hz) at room temperature (23□).

Results: The measured tan δ of tumor-implanted bones was significantly different from those of sham-operated bones ($p<0.001$). Storage modulus (E′) and loss modulus (E″) were also significantly different ($p<0.001$). At 0.1Hz, tan δ, E′, and E″ of the tumor-implanted bones were lower by 17%, 11%, and 13% respectively, as compared to those of the sham-operated bones. Across the range of frequencies between 0.001Hz and 70Hz, the slope that represents tan δ for the tumor-implanted bones was 17% lower than that for the sham-operated bones.

Conclusion: Results showed that viscoelastic response of tumor-implanted bones is lower as compared to that of sham-operated bones which may indicate the additive method of predicting osteolytic fractures. Based on these preliminary results, we are investigating the correlation between fracture risk and the change in viscoelasticity in a metastasized bone.

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INVESTIGATING THE EFFICACY OF ANTI-RESORPTIVE AND ANTI-CANCER TREATMENTS ON METASTASIZED BREAST CANCER BONE LOSS.

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Breast cancer patients have a strong tendency to develop bone metastasis, particularly in advanced stages. Bone metastasis, which leads to bone pain (initial symptom), bone fractures, nerve compression and hypercalcemia (with extensive bone lesions), effectively reduce the mobility and life expectancy of patients . We investigated the treatment responses of anti-resorptive (Ibandronate) and anti-cancer (Paclitaxel) in-vivo, which are believed to have a synergistic effect on the treatment of tumour induced osteolysis.

An experimental rat cancer model was employed in this pilot study, where a localized tumour growth was established. Malignant rat breast cancer cells (Walker 256 carcinosarcoma) were surgically implanted into the left femur of 20 female Sprague Dawley rats, while another 5 received sham operation. Of the 20 tumour-bearing rats, 5 were untreated (CANC group), 5 were administered with Ibandronate (IBAN group), 5 with Paclitaxel (PAC group) and another 5 with a treatment combination of Ibandronate and Paclitaxel (IBAN + PAC). The changes in bone geometrical and mechanical properties due to tumour-induced osteolysis were monitored using peripheral quantitative computed tomography (pQCT) and serum DPD (deoxypyridinoline) concentration at regular time intervals to observe the progress of bone resorption.

Localized tumour growth in the distal femur was found to be successfully induced in this rat model. After 30 days, the femurs were harvested and subjected to three-point bending tests to investigate mechanical property changes due to tumour induced osteolysis and to compare the relative effect of the different treatments. It can be shown that Ibandronate and Paclitaxel have a synergistic effect in-vivo and the drug combination provides a better efficacy than when either drug is used alone. This study also demonstrates the use of serum analysis and pQCT respectively,as useful tools for monitoring the biochemical and structural changes that accompany tumour-induced osteolysis.

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THE CHANGE OF INDENTATION VISCOSITY AND MODULUS IN TUMOR-BEARING RAT FEMURS

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Bone metastases are devastating diseases which caused increased morbidity. Breast and prostate cancer are tumors that most commonly metastasize to bone. Bone metastasis is characterized by the reduction and deterioration in bone quality. Bone has remarkable viscosity due to the viscoelastic nature of collagen fibers in bone matrix. Bone mineral density (BMD) is the current clinical tool to measure bone quality and predict fracture risk. However, incident of fractures were found to increase even though there is no changes in BMD. Hence, it has been acknowledged that the bone quality may be influenced not only by BMD but also by other factor like viscoelasticity. Thus, the aim of this study is to evaluate the changes in viscoelastic properties of tumor-bearing rat femurs using nanoindentation.

A tumor-bearing animal model was prepared with an injection of breast malignant cancer cells (Walker Carsinosarcoma 256) into one side's rat femur while the other side received sham operation. Nanoindentation testing

was performed at multiple locations across longitudinal sections of distal femurs that were harvested at 30 days after the surgery. Creep phenomena were observed as an increase in depth while holding the maximum load. With a representation of bone viscoelastic properties using two-element Voigt model, creep displacement-time curve was fitted to determine the elastic modulus and viscosity of rat femoral cortical bone.

Results showed that tumor-bearing femurs exhibit significantly lower indentation viscosity (η) and modulus (E) than sham-operated group ($p<0.05$), as hypothesized. We found that both tumor-bearing and sham-operated femurs group displayed a positive linear relationship between indentation viscosity and modulus. However, there is no significant difference in the correlation between cancer-induced and sham-operated rat femurs ($p>0.05$). Current results would be used to further evaluate the response of tumor-bearing metastasis to anti-cancer and/or anti-resorptive treatments for predicting risk of fractures in bone metastasis patients.

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DECREASED BONE TURNOVER DESPITE PERSISTENT SECONDARY HYPERPARATHYROIDISM DURING PROLONGED TREATMENT WITH IMATINIB

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Context : The tyrosine kinase inhibitor imatinib mesylate has an established role in the management of a number of malignant and proliferative conditions. Cross-sectional and short-term prospective studies have demonstrated secondary hyperparathyroidism during imatinib therapy, and variable changes in markers of bone turnover.

Objective : To determine the biochemical and skeletal effects of imatinib during long-term therapy

Design : 2 year prospective study.

Setting : Academic clinical research center

Patients or Other Participants : 9 patients with *bcr-abl* positive CML

Interventions : Imatinib mesylate 400mg/day.

Main Outcome Measures : Serum and urine biochemistry, markers of bone turnover, bone mineral density.

Results : Participants developed mild secondary hyperparathyroidism, with significant decreases in serum calcium and phosphate ($P<0.05$ and $P<0.0001$ vs baseline, respectively) and an increase in parathyroid hormone ($P<0.0001$ vs baseline). Biochemical markers of bone turnover demonstrated a biphasic response, with an initial increase in markers of bone formation being followed by a decrease in markers of both formation and resorption. Bone density at the lumbar spine increased (mean [95% CI] change from baseline 3.6% [1.6, 5.5], $p=0.003$) as did that at the total body (1.4% [0.2, 2.5], $p=0.065$), while that at the proximal femur did not change (-0.12% [-3.0, 2.7], $p=0.93$). Body weight and fat mass increased significantly ($P<0.0001$ vs baseline).

Conclusions : Long term treatment with imatinib leads to persistent mild secondary hyperparathyroidism. Despite this, bone turnover is decreased, and bone density is stable or increased. These results suggest that 2 years of therapy with imatinib is not associated with progressive biochemical abnormalities or skeletal harm.

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REDUCED PRE-DIAGNOSTIC 25-HYDROXYVITAMIN D LEVELS IN WOMEN WITH BREAST CANCER

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Background: Vitamin D may exert anticarcinogenic effects as v itamin D is known to increase differentiation and decrease proliferation in different cell types, including breast- and breast cancer-cells. In women with breast cancer, only few and conflicting clinical data are available on pre-diagnostic vitamin D levels.

Aim In a case-control analysis nested within a cross-sectional study, we determine whether risk of breast cancer is associated with pre-diagnostic plasma 25-hydroxyvitamin D (P-25OHD) levels and to what extent life-style characteristics known to influence vitamin D status affects risk of breast cancer.

Subjects and methods: we studied w omen without a prior history of breast cancer referred to a diagnostic mammography examination (n= 2,465). Cases were women diagnosed with an incident breast cancer (n=142). Controls were women not diagnosed with a breast cancer matched to cases on age, menopausal status, and time of year of blood sampling (n=420). Characteristics of cases and controls were assessed by a self-administrated questionnaire. We calculated r elative risk (RR) with 95% confidence interval (95%CI) for breast cancer by tertiles of P-25OHD. We adjusted for differences between cases and controls in life style characteristics known to influence vitamin D status.

Results: Cases had lower P-25OHD levels than controls. Compared with the lowest tertile of P-25OHD levels, risk of breast cancer was significantly reduced among women in the highest tertile (RR 0.52; 95%CI, 0.32-0.85). Risk estimates were similar in women with an oestrogen receptor positive and negative breast cancer. Use of vitamin D supplements, sunbathing frequency, and fish intake was associated with P-25OHD levels, but did not affect risk of breast cancer.

Conclusion: Risk of breast cancer is inversely associated with P-25OHD levels. Randomised controlled trials are warranted in order to assess whether a causal relationship exist.

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HUMAN MESENCHYMAL STEM CELLS(MSCS) TARGET OSTEOSARCOMA AND PROMOTE ITS GROWTH AND PULMONARY METASTASIS

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Osteosarcoma (OS) is the most common primary malignancy of bone and suffering patients often develop pulmonary metastases. Mesenchymal stem cells (MSCs) have been recently described to target many carcinomas. However, the interact between MSCs and OS has not been discussed. Here, we hypothesized that human bone marrow-derived mesenchymal stem cells (hMSCs) have a tropism for primary OS and thus play a role in its growth and metastasis. To test this, we first established an animal model of primary osteosarcoma in the tibia of nude mice using Saos-2 cells. Then we isolated and hMSCs from the bone marrow of one volunteer, labeled the cells with green fluorescent protein (GFP), then injected them into the nude mice bearing human primary OS xenografts by caudal vein. Eight weeks later, the GFP labeled hMSCs were found in the OS and lung tissue. The tumor volume was bigger and the pulmonary metastasis rate was higher in hMSCs injected group than in the non-treated group. Immunohistochemistry showed positive SDF-1 staining in the primary tumor tissue and CCL5 positive staining in pulmonary metastasis tissue in hMSCs injected group while the western blotting demonstrated the receptor CXCR4 expression in hMSCs and CCR5 expression in Saos-2 cells. The transwell assay showed that blockage of SDF-1 in condition medium of Saos-2 cells (Saos-2-CM) could repress the immigration of hMSCs stimulated by the Saos-2-CM. Moreover, blockage of CCL5 in conditioned medium of hMSCs (MSCs-CM) could restrain the migration and proliferation of Saos-2 cells stimulated by the MSCs-CM. Collectively, these data demonstrated that exogenous hMSCs could target OS, enhanced its growth pulmonary metastasis. SDF-1/CXCR4 and CCL5/CCR5 played an important role in mediating this interaction of hMSCs with Saos-2 cells.

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POTENTIAL ROLE OF PROTEIN DEACETYLASE SIRT1 IN METHOTREXATE CHEMOTHERAPY-INDUCED BONE AND BONE MARROW DAMAGE

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Severe bone damage is a known adverse effect of cancer chemotherapy. Our recent studies employing a rat model of an acute methotrexate (MTX) chemotherapy demonstrated that daily methotrexate injections at 0.75mg/kg for five consecutive days caused decreased trabecular bone in tibia as well as an increased adipocyte (AD) density in the bone marrow on day 9 post-treatment (Xian CJ, 2007). This correlated with reduced numbers of ALP-positive colony forming units-fibroblast (CFU-F) and increased differentiation of Oil-Red positive adipocytes when primary marrow collected from day 9 rats were grown ex vivo. Both in vivo and ex vivo assays suggest MTX induces systemic damage to the bone homeostasis and a change in bone marrow microenvironment. However a mechanism underlying the described chemotherapy-induced osteopenia and the potential osteogenesis-adipogenesis switch is poorly understood. Recently it has been found that in cell culture models, Sirt1 gene, a member of Sirtuin family of protein-deacetylases, functions as a switch between activation of osteogenesis via up-regulation of osteoblast markers and block of adipogenesis via inhibition of PPAR-γ (Bäckesjö CM, 2006, 2009). This instigates our analysis of possible effect of chemotherapy on Sirt1 expression. Real time RT-PCR analysis of bone samples at days 9 and 14 post the initial injection showed a decrease in the expression of Sirt1 in day 9 bone samples and recovery of its levels in day 14 samples compared to the controls. Interestingly, levels of Sirt1 expression closely mirrored the patterns of bone loss and a reduced pool of osteoprogenitor cells in the bone marrow and conversely the increased marrow fat content and ex vivo adipogenesis. Our preliminary study suggests that the Sirtuin proteins regulating multiple gene/protein targets could be well placed to be involved in systemic bone and bone marrow tissue responses observed after chemotherapy.

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VITAMIN D DEFICIENCY IS ASSOCIATED WITH ENHANCED TUMOUR GROWTH IN A MOUSE MODEL OF BREAST CANCER GROWTH IN BONE

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Secondary spread of breast cancer often includes dissemination of the primary tumour to the bone. The active metabolite of Vitamin D, 1,25-Dihydroxy-vitamin D (1,25(OH)2D3) plays a pivotal role in calcium homeostasis, regulation calcium storage/release from bone. In addition, it demonstrates local anti-proliferative effects in many tissue types, including breast cancer cells. We hypothesized that Vitamin D deficiency will promote more rapid breast cancer tumour growth in bone.

Using dietary restriction, we produced Balb/C nu/nu mice with severe Vitamin D deficiency, (plasma 25-Hydroxy-vitamin D3 levels of <20nmol/L versus levels >110nmol/L in vitamin D-sufficient mice) ($P<0.001$). Vitamin D deficient mice have increased tartrate-resistant acid phosphatase 5b (TRAcP5b) levels, indicating increased bone resorption. Vitamin D-sufficient and Vitamin D-deficient mice were injected intra-tibally with the human breast cancer cell line MCF-7, which produces mixed lytic and sclerotic lesions. Changes in the bone were followed by X-ray imaging. In addition, effects of 1,25(OH)2D3 on in vitro proliferation was assessed at concentrations of 10-9M to 10-7M. Nuclear Vitamin D receptor (VDR) expression was also evaluated in this cell line.

Vitamin D-deficient mice developed larger mixed osteolytic and osteosclerotic lesions compared to Vitamin D-sufficient mice. At days 10 and 14 after cancer cell inoculation, both groups exhibited similar lesion development. However, at days 21 and 28, Vitamin D-deficient mice had larger lesion areas of 1.38±0.11mm2 vs. 0.88±0.14mm2 and 1.71±0.11mm2 vs. 1.32±0.10mm2 respectively (mean±SEM, $P<0.01$). MCF-7 cells were markedly sensitive to

1,25(OH)2D3 in vitro, with 88.3% ($P<0.05$) suppression of proliferation at maximum inhibitory concentrations of 10-8M. VDR was stably expressed in MCF-7 cells, with 7-fold up-regulation by 1,25(OH)2D3.

Vitamin D deficiency was associated with accelerated tumour growth in nude mice. The mechanisms by which Vitamin D exerts its protective effects may include suppression of the bone resorption and/or direct growth inhibition of the tumour in bone.

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BONE MINERAL DENSITY (BMD) IN PATIENTS WITH GERM CELL TUMORS (GCT)

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Background: During recent decades the survival rate of patients with GCT has substantially improved. Consequently the long-term side effects of treatment of GCT have gained attention, including accelerated bone loss leading to increased risk of osteoporosis. Treatment-related bone loss is well recognized in breast and prostate cancer, but there has been little information in long-term survivors from other tumors.

Aims: Determine BMD and serum bone turnover markers in survivors from GCT.

Methods: BMD was measured by dual-energy X-ray absorptiometry in the lumbar spine and hips. BMD was classified as osteopenia (T score ranging from -2.5 to –1.0) and osteoporosis (T score less than –2.5).

C-terminal cross-linked telopeptides of type I collagen (CTX) were measured using Enzyme-Linked Immunosorbent assay (ELISA). Additionally serum total testosterone was measured.

Comparison was made with matched control data. Relationships between baseline characteristics (age, treatment type, and time from orchiectomy) and BMD were assessed using univariate and multivariate analysis tools.

Results: We included 479 patients in the study (21-76 yrs old, median: 39 yrs) who were treated for GCT since 1982. In this group, 436 pts (91,02%) were treated by unilateral orchiectomy and 43 pts (8,98%) by bilateral orchiectomy(OE). The further treatement was radiotherapy of retroperitoneal lymph nodes (RPLND) in 78 pts (16,28%), chemotherapy in 277 pts (57,83%), radiotherapy and chemotherapy in 11 patients (2,30%), the rest 113 pts(23,59%) did not received any adjuvant therapy. Median time since of OE was 6.5 yrs, average time was 8.1yrs.

Conclusions: We did not find any significant differences between GCP and matched control data regarding incidence of osteopenia and bone turnover marker, but the incidence of osteoporosis was considerably higher in GCP.

The incidence of osteoporosis appeared to increase with age and slightly correlated with time since OE, particularly after 10 years after OE. Type of therapy did not prove to have significant impact on the appearance of osteoporosis. Serum testosterone level did not correlate with BMD.

The long term survivor from GCP has significantly higher risk of osteoporosis than healthy matched population.

The preliminary data was published at ASCO 2007.

Category 8. Comparative Endocrinology

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STRUCTURE AND FUNCTION OF FUGU PARATHYROID HORMONE 1 (-34)

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Synthetic peptides of Fugu parathyroid hormone 1 (Fugu Pth1) were generated to study the structure and the activity of this fish homolog of human parathyroid hormone (hPTH). Three-dimensional nuclear magnetic resonance (NMR) was carried out on the N-terminal region of Fugu Pth1 to determine its structure and the differences between it and hPTH. We also compared it with the NMR structure for the human? PTH/PTH-related protein (PTHrP) receptor: hPTH1R (Mierke et al, 2007) and the possible interactions between hPTH1R and Fugu Pth1. The activity of Fugu Pth1(1-34) in

the signalling of PTH1R was also examined with both the human and the zefrafish receptor. Previously, we had shown that Fugu Pth1(1-34) is 5 to 10 times less potent than hPTH(1-34) in the mammalian adenylate cyclase system (Danks et al, 2003), but now we have a more extensive picture of the signalling systems it utilizes, including protein kinase A and protein kinase C. The structure of Fugu Pth1 and its signalling systems profile assist in understanding the evolution of parathyroid hormone and why the fish homolog and the mammalian receptor can act in a similar fashion to form new bone (McManus et al, 2008).

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(2) Danks, J.A., Ho, P.M.W., Notini, A.J., Katsis, F., Hoffmann, P., Kemp, B.E., Martin T.J. and Zajac, J.D. Identification of a parathyroid hormone in the fish, Fugu rubripes. J. Bone Miner. Res., 18:132

(3) McManus JF, Doust EA, Davey RA, MacLean HE , Ma C, Sims NA, Bouxsein ML, Glatt V, Zajac JD and Danks JA Intermittent Fugu parathyroid hormone 1 (1-34) is an anabolic bone agent in young male rats and

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TRABECULAR AND CORTICAL BONE CHANGES DUE TO LONG TERM VITAMIN D DEFICIENCY IN SENESCENT ANIMALS

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We have previously reported that while short-term vitamin D depletion in rats caused osteopenia in the distal femoral metaphysis, no changes were observed in the tibial cortices with regards to bone loss, geometry and strength. The question, however, of whether the effects of longer-term dietary vitamin D deficiency in adult rats impacts on cortical bone structure remains unclear. Hence, nine-month-old female Sprague-Dawley rats (n=5-6/grp) were pair-fed a semi-synthetic diet containing 0 or 500 ng/d of vitamin D_3 (D) with either low (0.1%) or high (1%) dietary calcium (LCa or HCa) for 6 months. At 15 months of age, animals were killed and femora retrieved for cortical and trabecular bone analyses. Cortical bone volume (CBV) at the mid-point of the femora (4mm segments) were analysed as well as the proximal and distal femoral metaphyses. CBV and the proximal metaphysis were analysed using 3D μCT scans (Skyscan) at a resolution of 12.6 μm/pixel. Distal metaphyses were analysed using 5-μm von Kossa-stained sagittal sections with standard histomorphometric techniques. Serum 25D and calcium levels ranged from 22(± 2.9)ηmol/L and 2.5(± 0.05)mmol/L respectively in the animals fed 0D/LCa diet up to 161(± 38.8)ηmol/L and 2.9(± 0.09)mmol/L in the animals fed 500D/HCa diet. Using regression analyses, positive relationships occurred between serum 25D levels and CBV (R^2=0.32, P<0.01), proximal femoral BV/TV (R^2=0.30, P<0.01) and distal femoral BV/TV (R^2=0.30, P<0.01). There was no significant association between serum calcium levels and bone volume measurements at any of the anatomical sites measured. However, in multiple regression analyses, the combination of serum calcium and 25D levels as determinants of cortical and cancellous bone volume, accounted for a greater proportion of the variation in bone mineral volumes (CBV R^2=0.65; proximal metaphysis R^2=0.57; distal metaphysis, R^2=0.58. Hence, longer-term vitamin D deficiency causes bone loss in cortical bone as well as in cancellous bone. Although vitamin D status is a major determinant of bone mineral content, serum calcium levels may contribute to the bone volume in this model. This dietary model is currently being used to further examine the effects on osteocyte density, apoptosis and mechanical strength of long bones.

A COMBINED TECHNOLOGICAL METABOLOMIC STRATEGY FOR THE IDENTIFICATION OF METABOLITES IN HUMAN URINE

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Advances in analytical chemical technologies including nuclear magnetic resonance spectroscopy (^{1}H-NMR), high performance liquid chromatography coupled to mass spectrometry (HPLC-MS) (Fig. 1), direct-infusion mass spectrometry (DI-MS) and gas chromatography coupled to mass spectrometry (GC-MS) allows for a rapid identification of metabolites in urine. Accompanied with "metabolomics" based data reduction using multivariate data analysis (MDA) including Principal Components Analysis (PCA) (Fig. 2) and Hierarchical Cluster Analysis (HCA) (Fig. 3), novel changes between phenotypic or metabolic states can be characterized in urine samples. As a proof of concept, 24 urine samples were analysed using NMR, LC-MS, DI-MS and GC-MS. Many metabolites were identified using databases and spectral deconvoluting software. A simple sample preparation protocol is presented for normalizing the water content in urine to the amount of creatinine present, without the need for derivatisation or complex chemical intervention. ^{1}H-NMR spectra identified 50 high to medium abundance metabolites, and LC-MS, DI-MS and GC-MS identified hundreds of both confirmed and putative metabolites. Many non-essential amino acids and organic acids were identified by all analytical platforms, but other compounds were solely identified by one technique. Non-targeted MDA of the 24 urine samples revealed similar metabolite differences between the four analytical techniques, which emphasizes the complementary nature of multi-technique approaches for biomarker discovery. The results presented in this study highlight 4 key points. Firstly, this study provides methodology for urine sample preparation, for analysis by NMR, HPLC-MS, DI-MS and GC-MS. Secondly, many well characterized and uncharacterized urinary metabolites can be identified using a multi-technique approach. Thirdly, this approach provides both complementary and additional information about urinary metabolites. Finally, MDA assists in revealing qualitative and quantitative information of potentially novel metabolites between different urine samples.

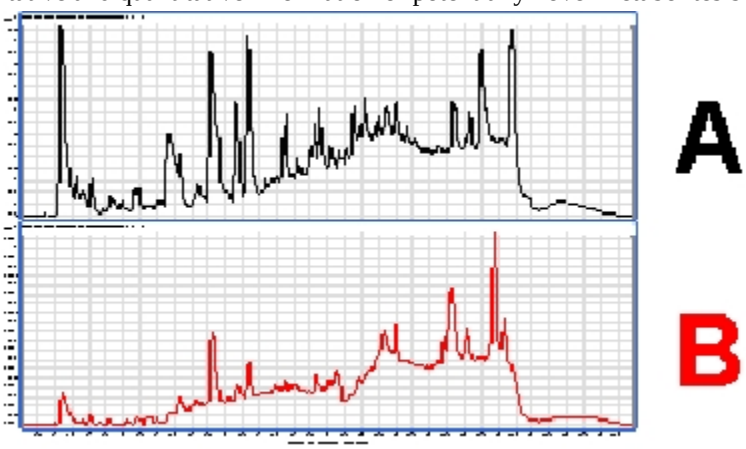

Figure 1. LC-MS Total Ion Chromatograms of two different urine samples A (black) and B (red).

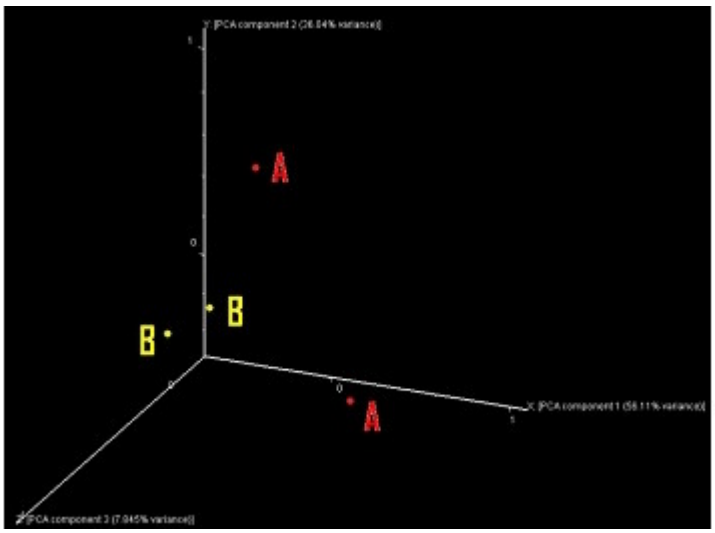

Figure 2. PCA scatter plot of urine samples A (red; n = 2) and B (yellow; n = 2) denoting the overall metabolic differences between the two samples.

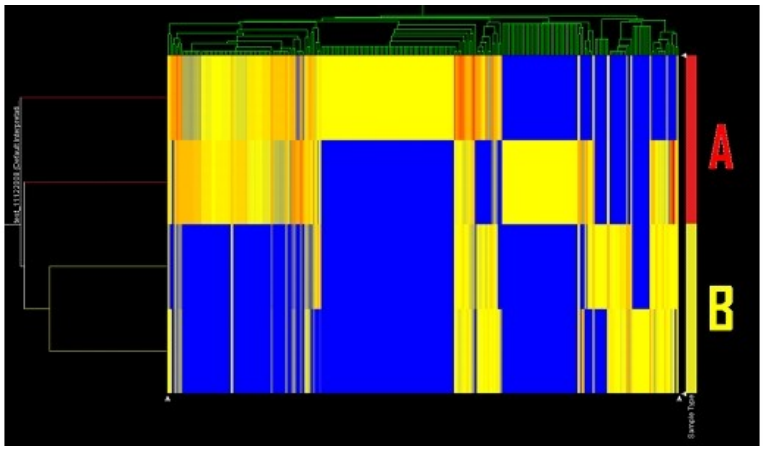

Figure 3. HCA of urine samples A (red; n = 2) and B (yellow; n = 2) illustrating metabolite differences between the samples on the basis of retention time and mass to charge ratio.

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EFFECT OF ANDROGEN ON AVIAN MEDULLARY BONE FORMATION

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Medullary bone is specific to female birds and plays an important role as a calcium reservoir for eggshell formation. The medullary bone appears just before the onset of egg-laying in bone marrow cavities of long bones, indicating that their formation is induced by the increasing amounts of estrogen secreted from mature follicles. It has been demonstrated that estrogen directly induces the medullary bone formation mediated by estrogen receptor. Androgen is also essential for medullary bone formation, collaborating with estrogen, and the medullary bone formation is easily induced by the administration of estrogen to mature male birds. However, it is unknown whether androgen directly stimulates medullary bone formation. Therefore, we tried to detect the localization of androgen receptors in medullary bone, and to clarify the role of androgen receptor in medullary bone formation.

For immunohistochemistry, medullary bone was dissected from femurs of mature female chickens, and paraffin sections were prepared. After antigen retrieval method using an autoclave treatment (citrate buffer, 10 mM at pH6.0), the localization of androgen receptors was detected with the primary antibody (rabbi polyclonal IgG antibody against androgen receptor; PG-21; Upstate) and the avidin-biotin complex method. Next, medullary bone formation was induced by the treatment of male Japanese quails with a single injection of estradiol valerate (2 mg/100 g body weight) (control group), and concomitantly, bicalutamide (3 mg/100 g body weight), androgen receptor antagonist, was administered intramuscularly to the quails for 7 day (experimental group). Thereafter, their medullary bone sections were stained with alucian blue, and the proportion (%) of the medullary bone to bone marrow cavity was calculated with Image-pro Discovery software.

Androgen receptors are localized in osteoblasts on medullary bone surface. However, osteoclasts and bone marrow cells do not represent the localization of androgen receptors. Medullary bone formation is induced by a single injection of estradiol, and the proportion represents 14.30% of bone marrow cavity. On the other hand, bicalutamide significantly inhibits the estrogen-induced medullary bone formation (down to 0.94%).

In conclusion, androgen receptor is present in osteoblasts but not in osteoclasts, and androgen directly stimulates osteoblastic medullary bone formation.

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IMPACT OF IL-1BETA-INDUCED UPREGULATION OF CALCIUM SENSING RECEPTORS ON L-AMINO ACIDS SENSITIVITY IN HUMAN CALCITONIN-SECRETING CELLS

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We have previously demonstrated that L-amino acids allosterically activate extracellular Ca2+-sensing receptors (CaR) in CaR-expressing HEK293cells and normal human parathyroid cells. In normal parathyroid cells, L-amino acids activate intracellular Ca2+ mobilization and suppress PTH secretion. In the current study, we have investigated the impact of enhanced CaR activation on the intracellular Ca2+ mobilization and calcitonin secretion from human TT thyroid cells under either control conditions or following exposure to hIL-1β to promote CaR expression (1). TT cells were cultured in F12-K nutrient medium with 10% FBS in the absence or presence of 100 ng mL-1 human interleukin 1β (hIL-1β) for 48 h. For the analysis of intracellular Ca2+ mobilization, TT cells were cultured on coverslips, loaded with the Ca2+-sensitive dye fura-2 AM (5 μM; 1.5 h) and then analyzed for sensitivity to elevated Ca2+ concentration or L-amino acids including L-Phe or L-Trp. For analysis of calcitonin secretion, TT cells were cultured in 24-well plates in the absence or presence of hIL-1β as above and then incubated with Ca2+ (0.5 to 1.5 mM) in the absence or presence of 10 mM L-Phe for various times (0 - 12 min). In fura-2 loaded cells under control conditions, increasing the extracellular Ca2+ concentration from 0.5 to 2.5 mM had little or no effect on intracellular Ca2+ and, in the presence of 2.5 mM Ca2+, only around 10% of cells responded to 10 mM L-Phe. After 48 h exposure to hIL-1β, however, there was a marked increase in sensitivity to extracellular Ca2+ and L-Phe. Under these conditions, around 20-30% of cells responded to 2.5 mM Ca2+ alone and, in the presence of 2.5 mM Ca2+, greater than 90% of cells responded to 10 mM L-Phe. In preliminary experiments of calcitonin release, control cells exhibited enhanced secretion at Ca2+ concentrations at or above 1.5 mM but there was no response to 10 mM L-Phe. In TT cells that had been exposed to IL-1β, however, there was enhanced sensitivity to elevated Ca2+ concentration and a threshold level was identified at around 1.0-1.2 mM. In addition, in the presence of 1.0 - 1.1 mM Ca2+, 10 mM L-Phe markedly stimulated calcitonin secretion. In analysis of CaR protein expression by western blotting, hIL-1β enhanced the expression of the mature 160 kDa form. The data indicate that a recognized hypocalcemic agent, hIL-1β, upregulates CaR expression in TT cells and, in consequence, there is enhanced sensitivity to extracellular Ca2+ and amino acids with respect to intracellular Ca2+ mobilization and calcitonin secretion. 1. Canaff L & Hendy GN. J. Biol. Chem. (2005) 280:14177 – 14188

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ESTRADIOL REGULATES RENAL KLOTHO THROUGH ESTROGEN RECEPTOR ALPHA

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Background: Klotho is a glycoprotein predominantly expressed in the kidney, parathyroid gland, reproductive organs and choroids plexus in the brain. Up-regulation of klotho by vitamin-D has been reported. Overlap in the expression pattern of estrogen receptors and klotho raises the potential for estrogen regulation of klotho expression. Methods: The mouse kidney distal convoluted tubule cell line, DCT, was cultured in DMEM supplemented with either 10% regular FBS or charcoal stripped FBS with or without added estradiol. To determine the effects of estrogen on klotho expression in vivo, we used wild type (WT, n=4) and aromatase deficient mice (ArKO, n=4) treated with estrogen (20ug/mouse 3x/week) or vehicle for 3 weeks. We also compared klotho expression in vehicle or estradiol treated ovariectomized estrogen receptor alpha (ERKOa) or beta (ERKOb) knockout mice to that of WT littermates. RNA and

protein were prepared from cells or kidneys for real time PCR and WB analysis, respectively. Results: Klotho protein was significantly higher in cells grown in csFBS compared to 10%FBS. Addition of estradiol at 10(-8)M in 10% csFBS restored klotho expression to the same level as cells grown in 10%FBS. There was significantly higher expression of klotho both at the mRNA and protein levels in ArKO animals compared to WT; however, ArKO mice treated with estrogen had WT levels of klotho. WT castrated mice treated with estradiol showed decreased levels of Klotho. Protein extracts prepared from kidneys of estrogen receptor alpha mice had higher levels than wild-type littermates. The suppressive effect of estradiol was lost in ERKOa animals. Estrogen receptor beta loss had no effect on Klotho expression. Conclusion: Estradiol suppresses klotho expression in the murine kidney through estrogen receptor alpha.

LATE POSTER SUBMISSIONS

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A DELAY IN CONSOLIDATION IS OBSERVED IN A HETEROZYGOUS CONDITIONAL BMP2 DEFICIENT MOUSE MODEL OF DISTRACTION OSTEOGENESIS

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Distraction osteogenesis (DO) is a surgical technique used to treat limb length discrepancies, limb deformities, long bone non-unions and bone loss due to trauma, infection or malignancies. One of the main limitations of DO is the long consolidation period required for the bone to heal. Different methods have been researched to accelerate the consolidation phase of DO, including the exogenous application of bone morphogenetic proteins (BMPs). BMPs are growth factors that are required in the bone developmental pathway. Although numerous studies have tested pharmacological doses of BMPs during DO, the physiological role of BMPs during DO still remains unclear. In this study, we investigated the physiological role of BMP2 during DO in heterozygous conditional BMP2 knockout mice.

Distraction osteogenesis was performed on the right tibia of eighty wild-type BMP2 fl/+ and heterozygous BMP2 fl/+ cre mice using a miniature version of the Ilizarov fixator. Mice underwent a latency period of 5 days, a distraction period of 12 days (distraction rate of 0.2 mm every 12 hours) and a consolidation period of 34 days. Distracted samples were collected from four time points: 11 days (mid-distraction phase), 17 days (end of distraction phase), 34 days (mid-consolidation phase) and 51 days (end of consolidation phase). Samples were studied using µCT, Faxitron x-ray, immunohistochemistry, histology, Real Time-quantitative PCR and biomechanical testing.

Results from this study showed that mice with a gene-dosage dependant reduction of BMP2 expression may be contained a delay in consolidation. µCT analysis revealed a statistically significant decrease in trabecular number and increase in trabecular separation at 51 days in the heterozygous mice. Immunohistochemical studies demonstrated decreased BMP2, BMP7, BMPR1a, ACTR1, ACTR2b expression in the heterozygous mice at 34 days post-osteotomy; which can account for the poor bone formation patterns observed during the consolidation phase of DO. Biomechanical testing of 51 day samples revealed a decrease in stiffness and increase in ultimate displacement in the heterozygous mice compared to the wild-type controls, corresponding to the the weaker consolidated bone of the heterozygous mice during this phase. Therefore, results from this study suggest that BMP2 exerts a significant physiological role during DO.

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OSTEONECROSIS OF JAWBONES IN TWO OSTEOPOROSIS PATIENTS TREATED WITH A NITROGEN-CONTAINING BISPHOSPHONATE (NBP): ATTEMPTS AT OSTEONECROSIS REDUCTION BY REPLACING NBP WITH ETIDRONATE (A NON-NBP)

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Among the bisphosphonates (BPs), the nitrogen-containing BPs (NBPs, such as zoledronate, risedronate, and alendronate) have anti-bone-resorptive effects (ABREs) that are much more powerful than those of the non-nitrogen-containing BPs (non-NBPs, such as etidronate and clodronate). In the last few years, a thousand or so cases of osteonecrosis of the jawbones (ONJ) have been suspected of being associated with the administration of NBPs. However, the mechanism underlying the osteonecrosis remains unclear, and there are no effective therapeutic methods. Since NBPs accumulate in the hydroxyapatite within bone, our fear is that more cases will come to light if NBPs continue to be used as they are now. The situation is made more complex by (a) NBP-associated ONJ developing even after a pause in the NBP-treatment, and (b) ONJ sometimes appearing long after discontinuation of NBP-therapy (in some cases, as long as 12 months after). Interestingly, and importantly, few ONJ cases have been reported in patients treated with non-NBPs such as etidronate and clodronate. We previously reported that in mice: (i) etidronate (a non-NBP), when intraperitoneally co-administered with alendronate (an NBP), competes against the NBP for binding to bone hydroxyapatite and (ii) etidronate can reduce the inflammatory effect of alendronate (Funayama et al. Calcif Tissue Int 76:448-457, 2005). These findings led us to expect that etidronate might eliminate an NBP that had already accumulated within bone, and that etidronate might therefore be useful as a substitution drug in NBP-treated patients at risk of ONJ. Here, we describe the apparent effectiveness of such etidronate-replacement therapy in two NBP-treated patients with ONJ and/or osteomyelitis. They had been receiving oral risedronate (for 55 or 66 months) as treatment for osteoporosis. Bone scintigraphy of the mandible revealed a marked accumulation of 99mTc-HMDP. The risedronate treatment was discontinued, and treatment with oral etidronate (200 mg/day) was begun according to the standard prescription (i.e., two weeks• f ingestion and three weeks• f rest). Within 3 weeks of the start of the treatment, pain and pus had disappeared. In case-1, bone-scintigraphy images revealed shrinkage of the inflamed area of the mandible. We discuss the rationale for this therapeutic strategy. (bone-scintigraphy images in case-2?)

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CHANGES OF GENE EXPRESSION PROFILING AND PATHWAYS RELATED FAT METABOLISIM IN FEMORAL HEAD OF STEROID-INDUCED OSTEONECROTIC RATS

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Objective :To understand the changes of gene expression profiling and pathways related fat metabolism in femoral head of steroid-induced osteonecrotic rats in the contribution to pathgenesis of steroid-induced osteonecrosis.

Methods : We applied lippolysccharide(LPS) and methylprednisolone(MPSL) to prepare a rat model of steroid-induced osteonecrosis(SIO).Rats were divided into two groups: normal(N) group and model(M) group. All rats sacrificed after 6 weeks. Then we applied genome wide cDNA microarry technology to analyze genes expressed in femoral head of two groups and also serum levers of cholesterol(TC) were measured in two groups.

Results: (1) There were total 111 expession genes which changed by a minimum of two-fold and also it was found that 18 pathways had significant changes in these differential genes by molecule annotation system molecule annotation system (MAS) analysis in femore head of model group compared with normal rats.

(2) There were 6 upregulated genes related fatty acid metabolism(FAM) pathway(Acadl,Cpt2,Acaa2,Acat1,Acsl1,Hadhb), 2 upregulated genes related fatty acid elongation in mitochondria(FAEM)(Acaa2,Hadhb),3 upregulated genes related adipocytokine signaling pathway (ACK)(Cpt2,Prkaa2, Acsl1)et al in femore head of model group compared with normal rats.

(3) Serum levels of cholesterol significantly increased 21.2% in model group compared with normal group(1.754 ± 0.264 mol/L vs 1.447 ± 0.142 mol/L ,p<0.00 1).

Conclusions :The data suggest that the upregulated genes related fat metabolism in their pathways of femoral head of steroid-induced osteonecrotic rats might paly an important role in promoting the synthesis of fatty acid and cholesterol and fat metabolism could be mediated by FAM, FAEM and ACK pathways. The changes of multiple gene expression and that numerous pathways could play major roles in steroid-induced osteonecrosis pathogenesis.

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ANALYSIS OF CIRCULATORY FGF23 PROTEIN

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FGF23 is a physiological humoral factor regulating phosphate and vitamin D metabolism. Previous in vitro studies indicated that a part of FGF23 protein is proteolytically cleaved between 179Arg and 180Ser by subtilisin-like proteases, and only full-length FGF23 has a biological activity to reduce serum phosphate and 1,25-dihydroxyvitamin D levels. However, it is currently unknown how much of circulatory FGF23 protein is present in intact and processed forms in vivo and whether this ratio changes with impairment of renal function. There are several assay methods for FGF23. Intact FGF23 assay (Kainos) detects only full-length uncleaved FGF23. In contrast, C-terminal assay (Immutopics) recognizes both full-length and processed C-terminal fragment of FGF23. Therefore, we compared plasma FGF23 levels measured by these two assays in patients with tumor-induced osteomalacia (TIO) and end stage renal disease (ESRD) undergoing hemodialysis. In addition, we estimated the quantity of full-length and processed C-terminal fragment of FGF23 by immunoprecipitation and Western blotting using an antibody against the C-terminal portion of FGF23.

All cases showed very high FGF23 levels by both assays. In 30 patients with ESRD, FGF23 was 5917.8 +/- 1350.6 pg/ml (mean +/- SE, reference range 10 - 50 pg/ml) and 6525.2 +/- 1296.3 RU/ml (reference range < 150 RU/ml), by full-length and C-terminal assay, respectively. In 3 TIO patients, it was 2555.7 +/- 1782.4 pg/ml and 4277.8 +/- 3346.5 RU/ml. There was a strong positive correlation between FGF23 levels measured by these two assays. In addition, the relationships between FGF23 levels measured by these two assays were similar in patients with TIO and ESRD. Western blotting indicated that about 30% of FGF23 is present in the processed form and this ratio is not different between patients with TIO and ESRD. These results indicate that a certain amount of circulatory FGF23 is present in the processed form and this processing is not affected by the impairment of renal function. Therefore, the high level of FGF23 by C-terminal assay in patients with ESRD is not derived from the accumulation of processed C-terminal fragment of FGF23.

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COMPARISON OF CORTICAL BONE THICKNESS, POROSITY, AND DENSITY IN PRE- AND POSTMENOPAUSAL WOMEN MEASURED BY HR-PQCT AT THE DISTAL RADIUS AND TIBIA

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Reduced cortical thickness (Ct.Th) and increased cortical porosity (Ct.Po) have been documented in menopause [1] and linked to increased risk of fracture. The purpose of this study was to use high resolution peripheral quantitative computed tomography (HR-pQCT) on a population-based sample to compare Ct.Th, Ct.Po and cortical density (Dcort) between premenopausal women with normal bone mineral density (DXA FN T-score > -1) and postmenopausal women with normal, osteopenic, and osteoporotic bone mineral density. Automated cortical segmentation procedures [2], validated with µCT measurements, were used to obtain direct measurements of Ct.Th and Ct.Po. The analysis was applied to distal radius and tibia HR-pQCT scans from the Calgary cohort of the Canadian Multicentre Osteoporosis Study (CaMos). We used analysis of variance to compare cortical bone outcomes between 51 premenopausal normal, 72 postmenopausal normal, 101 postmenopausal osteopenic, and 11 postmenopausal osteoporotic women. At both the radius and tibia we found that postmenopausal women (all groups) had higher Ct.Po (3.6 to 13.2%, $p<0.001$) and lower Ct.Th (-4.8 to -32.6%, $p<0.001$) than premenopausal women. Dcort was also significantly lower in all postmenopausal

women compared with premenopausal women (-5.7 to -22.4%, p<0.001). Osteopenic and osteoporotic postmenopausal women both had higher Ct.Po (2.0 to 7.6%, p<0.001), lower Ct.Th (-12.2 to -29.2%, p<0.001), and lower Dcort (-6.2 to -15.7%, p<0.001) than the normal postmenopausal women. Postmenopausal osteoporotic women had 4.7% higher Ct.Po (p=0.005), -17.8% lower Ct.Th (p=0.006), and -8.5% lower Dcort (p<0.001) than the osteopenic women at the distal tibia. At the distal radius, only Ct.Po was significantly greater in osteoporotic women (3.9%, p=0.007). The morphological results in our population-based sample are consistent with previous HR-pQCT studies [3], and these results provide new data about the cortical porosity of the bone in pre- and postmenopausal women. The functional importance of these differences in cortical bone microstructure, as they relate to HR-pQCT estimates of bone strength, is currently under investigation.

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ALENDRONATE + VITAMIN D THERAPY OR REFERRED CARE IN OSTEOPOROTIC WOMEN: RATIONALE AND DESIGN, INCLUDING MEASUREMENT OF PHYSICAL FUNCTIONS, FALLS, AND POSSIBLE GENETIC MARKERS

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AIM: Vitamin D is required for bone strength and also acts on muscle function. Vitamin D insufficiency is prevalent, and often overlooked by physicians. A planned study and its extension will examine the effects of a single tablet containing the bisphosphonate alendronate 70 mg plus vitamin D_3 5600 IU (ALN+D) compared with referred care on serum vitamin D and BMD. Falls physical function, and genomic sampling will be exploratory endpoints.

METHODS: In an upcoming international, randomized, controlled trial of 6 months with a 6-month extension (under the same treatment assignments), approximately 800 women (>=65 years of age, osteoporotic, at increased risk of falls, with baseline 25(OH)-vitamin D of 8 to 20 ng/mL) will either receive ALN+D weekly or be referred to their primary care physicians (who are not investigators in the trial) for one of the usual osteoporosis therapies. Women in the ALN+D group with <=1000 mg daily calcium intake at baseline will receive 500 mg elemental calcium/day. The primary endpoint will be proportion of patients with serum 25(OH)-vitamin D<20 ng/mL. Secondary endpoints will include bone turnover markers. Exploratory endpoints will include fall event rate, the Short Physical Performance Battery (SPPB), and the relationships among genotype, RNA expression, total body composition, and SPPB. Falls will be reported by patients to their study site. Fall case report forms will include 15 questions concerning detailed description, location, and outcome of the fall. Falls due to fragility, but not due to a syncopic event or external force, will be included for data analysis. Fall case report forms will be adjudicated by an independent committee, blinded to patient-treatment group. Safety will be monitored.

CONCLUSION: This study may demonstrate relationships among osteoporosis/vitamin D therapy, falls, physical function, and molecular/genetic information.

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BASELINE BMC EXPLAINS ONLY 38 PERCENT OF GAIN IN BMC BETWEEN 5 AND 7 YEARS OF AGE IN NEW ZEALAND CHILDREN

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Children with large skeletons might be expected to gain more bone mineral than those with smaller bones. The present study was undertaken to determine to what extent baseline bone mass influences subsequent gain in BMC in healthy prepubertal children. Heights and weights were determined and total body BMC and body composition were measured using DXA (Lunar DPX-L) in 150 children participating in the FLAME birth cohort study. At 5 years of age girls (n=57) had 614.4 (86.5)g and boys (n=93) 658.2 (90.9)g of bone mineral content. Mean (SD) 2-year gains in the girls and boys respectively were: BMC (g) 205 (41) and 220 (49), bone area (cm2) 206 (40) and 212 (46), height (cm) 12.2 (1.3) and 12.1 (1.4), weight (kg) 5.4 (1.9) and 4.7 (1.4), lean mass (kg) 3.67 (0.73) and 3.95 (0.86), and fat mass (kg) 1.24 (1.64) and 0.39 (0.84). Deviations from the expected values for BMC gain were seen throughout the range of

baseline bone mass at 5 years of age. Baseline BMC explained 38% of the 2-year BMC gain with a further 14.8% (girls) and 29.0% (boys) being explained by deviations from expected gains in height and weight. Changes in body composition also influenced BMC increments. Deviations from expected gain in lean mass explained 12.0% (girls) and 22.6% (boys) while deviations from expected gain in fat mass explained only 7.8% (girls) and 1.6% (boys) of the 2-year BMC change. We conclude that although baseline skeletal mass influences the magnitude of gain in bone mineral, variations in anthropometric growth and body composition change (particularly lean mass) also make important contributions to BMC gains at this young age.

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ANALYSIS OF THE BONE MARROW STROMA IN AN IN VIVO MODEL OF HIGH BONE TURNOVER

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Expansion of a population of fibroblastoid cells in the bone marrow (BM) is typical of hyperparathyroidism. An expansion of similar cells was also observed in the BM of transgenic mice expressing a constitutively active PTH/PTHrP (PPR*Tg) in mature osteoblasts, and was fully abrogated by treatment with osteoprotegerin. The goal of this study was to characterize these fibroblastoid cells in PPR*Tg mice, and to investigate whether they shared any feature with cells of the inflammatory stroma.

For this purpose, PPR*Tg mice were crossed with a transgenic mouse line which expresses Green Fluorescent Protein (GFP) in mature osteoblasts (Col1GFP) in order to generate PPR*Tg/Col1GFP mice. Majority of the fibroblastoid cells in PPR*Tg/Col1GFP mice did not express GFP, which indicates that they were not mature osteoblasts. In situ hybridization analysis revealed that a variety of osteoblast markers were heterogeneously expressed by these cells. Collectively, these data indicate that the fibroblastoid population in the BM of PPR*Tg mice was mainly contributed by immature cells at early stages of osteoblast differentiation. Flow cytometry analysis of BM cells isolated from PPR*Tg/Col1GFP and Col1GFP mice, respectively, revealed the presence of a population that expressed both GFP and CD45, a pan hematopoietic cell-surface marker, and was thus phenotypically similar to “fibrocytes”. Fibrocytes are collagen-producing cells of hematopoietic origin, and have been identified in wounds and pathological fibrosis. Notably, number of these fibrocyte-like cells was significantly increased in the BM of PPR*Tg mice.

In conclusion, our findings indicate that: 1) expression of a constitutively active PTH/PTHrP receptor in mature osteoblasts leads to expansion of immature cells of the osteoblast lineage with mechanisms that are likely to be indirect and require osteoclast activity; 2) fibrocyte-like cells, which are typical of the inflammatory stroma, are also present in the normal BM and their number is increased in PPR*Tg mice.

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ASSOCIATION OF BONE MINERAL DENSITY AND BIOCHEMICAL MARKERS IN HEALTHY YOUNG VOLUNTEERS FROM NORTH INDIA

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Background. Since Indians have highest prevalence of low bone mass and lower bone mineral contents at hip and lumbar spine as determined by dual X-ray energy absorptiometry, which is a surrogate marker of treatment efficacy that has been widely used in clinical trial. The purpose of this study was to investigate the association of four biochemical markers of bone turnover with whole body BMD and at all three sites. High levels of bone resorption markers are associated with increased risk of osteoporotic fractures. A very high level of the bone turnover marker (T score >3) is suggestive of other metabolic bone disease. The combination of BMD and bone turnover measurement allows the identification of young subjects at a much higher risk for fracture.

Methods. Fifty one healthy subjects in the age group of 20 to 35 were taken. BMD was measured at the lumbar spine (LS) and femoral neck (FN) and forearm using DXA (GE Lunar, WI, and USA). Anthropometric measurements included height, weight, BMI and waist hip ratio (WHR). Estimation of Bone turnover markers included the levels of serum bone-specific alkaline phosphatase (sBAP), serum type I collagen cross-linked C-terminal telopeptide (sCTx), serum Osteocalcin and Parathyroid hormone (PTH)using standard ELISA kits.

Results. Out of fifty-one healthy volunteers only eight (15.68%) had normal BMD (6 males & 2 females), Seven (13.73%) were osteoporotic (5 women & 2 men) and thirty-six (70.59%) were osteopenic (20 males & 16 females).PTH negatively correlated with Total BMD and BMC, r=0.290, P<0.039 and r= 0.292, P< 0.038 respectively. Serum Osteocalcin had a positive corrlelation with BMI r=0.283, P<0.007.Serum Crosslaps showed a positive corrlelation with Total bone area (r= 0.373,P<0.007),Hip BMD(r= 0.333,P<0.017) and Forearm BMD(r= 0.357,P<0.010).There was a significant increase in serum PTH, levels in normal versus osteoporotic groups (P<0.004) and normal vs osteopenic groups.(P<0.030).We found no significant correlations with (sCTx), (sBAP) and sOsteocalcin fracture risks.

Conclusion. Patients with low BMD or high bone turnover marker values would be at risk for osteoporosis and warrant preventive measures with antiresorptive agents. High levels of bone resorption markers are associated with an increased risk of osteoporotic fractures.

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BMD AND BONE VOLUME FRACTION OF ENTIRE HUMAN VERTEBRAL BODIES EXAMINED BY DXA AND MICRO-CT

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The evaluation of fracture risk of patients is usually done using dual-energy X-ray absorptiometry (DXA) of the lumbar spine. Anterior-posterior (AP) projections are performed, with areal bone mineral density (BMD) measurements calculated within the whole vertebral body (L2, L3). However, the bone distribution and microstructure, and thus bone strength, might vary within the vertebra. Thus, subregional BMD measurements using lateral DXA scanning modality might be informative about fracture risk. Nowadays, X-ray micro-computed tomography (micro-CT) allows three-dimensional structural characterization of entire bone segments, non destructively and at high resolution. Aim of this study was to measure the BMD by lateral DXA in three subregions of the L2 human vertebra, and to compare it with measurements of bone volume fraction (BV/TV) in analogous subregions obtained by micro-CT.

Eight human cadaver spines (age range 61-91 years) immersed in a water bath were scanned by DXA in the AP and in the lateral projections. Then, subregional areal BMD analysis was done in the lateral projection of the L2 vertebra, with the examination area divided into three subregions (superior, central, inferior). The L2 vertebrae were then dissected and entirely scanned by micro-CT (18 μm pixel size). The micro-CT volume of interest comprised the trabecular bone of the entire vertebral body, and was divided via software into three equal subregions (superior, central, inferior), over which then analysis of the BV/TV was done.

Significant differences were found between the subregions (one way ANOVA for repeated measures), with BMD and BV/TV having higher average values in the inferior subregions than in the superior subregions ($p<0.05$). In the central subregion, the linear regression “BV/TV vs. BMD” had a high coefficient of determination ($R2=0.80$, $p<0.01$). While the BMD measured laterally over the whole vertebrae was significantly related to the total BV/TV ($R2=0.59$, $p<0.05$), the BMD measured in the AP direction was not ($p=0.33$).

These preliminary results suggest that, in contrast to AP BMD measurements, lateral BMD measurements in the L2 vertebra are significantly related to trabecular BV/TV. In particular, subregional BMD measurements are highly related to trabecular bone volume fraction in the central part of the vertebral body. These findings support lateral DXA examination as a valuable modality for improving the evaluation of vertebral fracture risk.

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VALIDITY OF USING A LINEAR MICRO-FINITE ELEMENT MODEL TO PREDICT TRABECULAR BONE APPARENT MECHANICAL PROPERTIES: COMPARISON WITH A NON-LINEAR MODEL AND EXPERIMENTAL DATA

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INTRODUCTION Micro-finite element (μFE) analysis is a popular tool for determining trabecular bone mechanical properties. Linear models have been shown to accurately predict areas of micro-damage and micro-fracture [1] ; with recent models attempting to predict apparent level yield stress and strain by incorporating material nonlinearity, but at the cost of increased computation time.

The objective of this study was to assess the capability of both linear and non-linear material models to predict apparent level mechanical properties. Trabecular bone specimens (1 cm3), taken from vertebrae of 12 human cadavers (mean (SD) age 69.25 (11.2) years), were imaged in a μCT scanner at 15μm resolution prior to undergoing uniaxial compression testing to 10% apparent strain.

METHODS Baseline image data were used to construct μFE models. Image data were resampled to 40μm resolution to ensure sufficient numerical convergence & minimise computation time; resultant FE meshes comprised on average 390,000 nodes.

μFE models were subjected to quasi-static loading to 5% apparent strain using both linear and non-linear material models. The linear analysis assumed a homogenous linear isotropic material model. The effective tissue modulus was back-calculated to match the apparent modulus obtained during testing. Non-linear analyses used a bilinear constitutive model with asymmetric yield strain and tangent modulus set to 5% of initial tissue modulus [2] .

RESULTS Due to the assumed fully-elastic behaviour failure stress could not be predicted for linear models; however at 3% apparent strain, average localised tissue strains were 2.84%, which is significantly larger than reported strain levels for trabecular tissue. For the nonlinear model, yield stress was underestimated; however regions of high stress or failed elements correlated with the same regions identified using the linear model.

DISCUSSION Whilst linear models are unable to predict apparent yield stress, they are useful for determining regions at risk of fracture. Incorporating non-linear material properties allows for a more realistic model, however at the cost of significantly increased computation time. Further investigation is required to determine non-linear behaviour of trabecular bone at the tissue level.

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COMPLIANCE WITH WEEKLY ALENDRONATE AN OUTPATIENT BONE CLINIC STUDY

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The treatment of osteoporosis with alendronate requires long term adherence to be effective. The purpose of this study was to determine weekly adherence to alendronate in patients with osteoporosis attending a specialist outpatient metabolic bone clinic. Our study included 112 consecutive patients treated for osteoporosis with alendronate that attended the clinic between June 2005 and June 2006. 85% of patients were female and the average age was 74 years old (range 43 to 90). All patients were given written instructions on how to take alendronate. Compliance at twelve months following the commencement of therapy was assessed at subsequent clinic visits or from other hospital visits or admissions included in the patient's clinical records. 67 (59.8%) of patients were taking alendronate for at least twelve months. 13 (10.7%) patients discontinued alendronate within twelve months and there was no available follow up data for 32 (28.6%) of patients. Of those that discontinued within twelve months, 8 (61.5%) experienced adverse events, 2 (15.4%) were deceased and 3 (23.1%) discontinued for other reasons. Of the patients that discontinued due to adverse events, 4 (50%) experienced gastrointestinal symptoms and there were 1 of each of bone pain, jaw pain and migraine. Limitations of the study include the relatively large number of patients with insufficient follow up data (28.6%), and the use of patient hospital records as a mechanism of determining compliance. In this study the worst case adherence rate with alendronate at twelve months is 59.8%, and the best case is 83.8% compliance. The main reason for discontinuation was the development of adverse events, particularly gastrointestinal symptoms. This study has highlighted the importance of following up patients on long-term alendronate therapy and providing clear written instructions to ensure optimal compliance.

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ASSOCIATION STUDY OF CYP3A5 GENOTYPE WITH BONE MASS IN KOREANS

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Osteoporosis is a disease that is strongly influenced by genetic factors. Low bone mass and high bone turnover are highly related to sex hormones. Polymorphism of the CYP3A5 gene is known to influence the functionality of the CYP3A5, the second CYP3A family member in the adult human liver , which can metabolize sex hormones. We, therefore, analyzed t he full extent of the CYP3A5 genetic polymorphism in 194 unrelated Koreans and evaluated the association of the CYP3A5 genotype with bone mineral density (BMD) in 2,178 women aged 40-79 years old. The most frequent single nucleotide polymorphism (SNP) was 6986A>G, which is responsible for CYP3A5*3. The next most frequent SNP was 31611C>T. Haplotype analysis using detected SNPs revealed that the most frequent haplotype was *3A (frequency: 0.724), followed by *1E (0.211), *3C (0.034) and *1A (0.023). CYP3AP1, CYP3A5*6, or *7 were not detected in our study. In 2,173 subjects, 62.7 % possessed the CYP3A5*3/*3 genotype which causes an aberrantly spliced mRNA with a premature stop codon and thus produces a non-functioning protein . A BMD difference, however, was not observed in women having whole CYP3A5 activity compared to women having deficient CYP3A5 activity, indicating no significant influence of CYP3A5 on bone metabolism. Our data suggest that the metabolic difference of CYP3A5 genotype may be counterbalanced by the major CYP3A such as CYP3A4.

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ANALYSIS OF BONE MINERAL DENSITY AND OSTEOPOROSIS RISK IN MANAWATU, NEW ZEALAND

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The 2006/07 New Zealand Health Survey estimates ~2.9% (or 90,000 people) of the NZ adult population are diagnosed with osteoporosis, with the incidence increasing with age. In our study we aimed to describe the population of the Manawatu region only.

Data was obtained from the only service provider for DXA (Lunar DPX-IQ Densitometer) within the Manawatu region. Baseline data from initial scans for females and males (>18 years of age), between the period of November 1996 to January 2008, were used for this study. The results, stratified for age and gender, are summarised in Table 1, and highlight the loss of BMD with age through deceasing T- and Z-scores, and increasing prevalence of osteoporosis and osteopenia.

Within the study population 2219 females reported a total 2717 fractures at the time of the baseline scan. Forearm fractures were the most common with 1265 fractures, followed by spine (685), and femur (340). 251 males reported a total of 304 fractures, with forearm fractures also the most common fracture (122), then spine (104) and femur (45).

Certain disease states and medications are known to increase risk of bone loss. Medication was reported when reporting for DXA scan. Corticosteroid use was the most common, with 954 females and 161 males reporting usage. The prevalence of other conditions reported included hyperthyroidism (343 females and 23 males) and renal disease (214 females and 28 males).

The incidence of osteoporosis in our population was much higher than previously reported for New Zealand, but this could be a reflection of the small and confined population studied. Further work is being done to assess patterns of bone loss and successfulness of intervention or treatment.

Table 1. Data are shown as mean ± SD.

	Females			Males		
	18-29yrs	30-54yrs	55+yrs	18-49yrs	50-69yrs	70+yrs
BMI (kgm^{-2})	23.0 ± 4.1	25.6 ± 5.2	26.1 ± 5.0	25.0 ± 5.5	26.5 ± 4.9	25.6 ± 4.6
Spine (n)	125	1961	5154	114	363	332
L2-L4						
T-Score	-0.22 ± 1.41	-0.05 ± 1.50	-1.20 ± 1.73	-0.11 ± 1.46	-0.64 ± 1.65	-1.00 ± 1.97
Z-Score	-0.18 ± 1.26	0.20 ± 1.48	0.48 ± 1.67	-0.18 ± 1.53	0.16 ± 1.57	0.24 ± 1.75
Osteoporosis %*	10.1	4.5	22.0	4.8	10.7	21.1
Osteopenia %*	22.5	21.2	34.0	27.2	34.7	27.7
Femur (n)	138	1967	5066	115	354	327
Total Femur						
T-Score	-0.46 ± 1.61	-0.16 ± 1.27	-1.31 ± 1.43	0.00 ± 1.31	-0.58 ± 1.31	-1.53 ± 1.57
Z-Score	-0.28 ± 1.31	0.05 ± 1.17	-0.14 ± 1.14	-0.12 ± 1.27	-0.08 ± 1.13	-0.49 ± 1.30
Osteoporosis %*	4.0	2.6	20.9	2.6	6.8	28.1
Osteopenia %*	17.5	22.8	38.3	18.3	31.1	37.3

* Osteoporosis = T-score ≤ -2.5; Osteopenia = T-score < -1 and < -1 and > -2.5

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FURTHER UNDERSTANDING FOR THE CAUSE OF BDA1 (BRACHYDACTYLY TYPE A1), THE CENTURY PUZZLE IN GENETIC HISTORY

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Brachydactyly type A-1 (BDA-1; MIM 112500), characterized by shortening or missing of the middle phalanges reported by Farabee in 1903, is the first recorded example of a human anomaly with Mendelian autosomal dominant inheritance and has been quoted in many genetic or biological textbooks. Fortunately, in our studies we successfully map the BDA-1 locus within an 8.1cM interval on chromosome 2q35-36 and then find three mutations of IHH (Indian hedgehog) are the cause for BDA-1.

In our following in vitro and in vivo studies, we use assays in cells and chick embryos, and gene targeted mice to show that a BDA1-E95K mutation in IHH (IHHE95K) impairs interaction between IHH with cell-surface transducers (PTC1) and modulators of HH signaling (HIP1), affecting the potency and range of signaling. In the mouse model that recapitulates the E95K mutation, homozygous IhhE95K mice display a classic BDA1 phenotype, with delayed endochondral ossification in the growth plate. As a result, we demonstrate the dominant E95K mutation in IHH causes digit abnormalities with occurrence of altering the capacity and range of signaling in vivo.

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VITAMIN D DEFICIENCY IN BREASTFED INFANTS

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Purpose: Vitamin D deficiency is a public health problem in many countries. There has been a reappearance of rickets from vitamin D deficiency in recent decades as a result of multiple factors. One of the factors is breast feeding. The purpose of this study was to describe the clinical presentation of rickets in breastfed infants.

Methods: Retrospective review of patients presenting to Ajou University hospital between 2003 and 2008 with rickets caused by vitamin D deficiency during breast feeding.

Results: Seventeen patients(10 boys and 7 girls) were diagnosed with vitamin D deficiency. There were six in the asymptomatic and eleven in the symptomatic patients. The mean age of the patients was 8.5 ± 0.5 months. The mean 25-hydroxycholecalciferol was 3.55 ± 1.88 ng/mL. 25-hydroxycholecalciferol levels were below 5 ng/mL in 13

patients. The mean serum alkaline phosphatase was 765.53 ± 563.9 IU/L, the mean intact parathyroid hormone was 231.6 ± 225.7 pg/mL. All except 3 patients were showed cupping and fraying of metaphysis.

Conclusion: Breast feeding is associated with increased risk of rickets. We recommend vitamin D supplementation of all breastfed infants to prevent rickets. Supplementation should begin within the first 2 months of life. Also, we hope to initiate further research and debate about guideline of vitamin D supplementation.

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THE EARLY ONSET OF CRANIOSYNOSTOSIS IN AN APERT MOUSE MODEL

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Activating mutations of FGFRs1-3 cause craniosynostosis (CS), the premature fusion of cranial bones, in man and mouse. Past analysis of a mouse model of the FGFR2 (Ser252Trp) Apert syndrome mutation suggested that increased apoptosis was the cause of suture fusion, beginning post-natally. We have reassessed coronal suture fusion in this mouse model, and provide the first detailed account of the process of embryonic coronal suture fusion in a mouse CS model. We find that the critical event of CS is the early (E13.5) loss of basal suture mesenchyme as the osteogenic fronts, expressing activated Fgfr2, unite to form a contiguous skeletogenic membrane. A mild increase in osteoprogenitor proliferation precedes but does not accompany this event, and apoptosis is insignificant. Bilateral coronal suture fusion occurs by E16.5. The more apical coronal suture initially forms appropriately but then undergoes fusion, at a slower rate, accompanied by a significant decrease in osteoprogenitor proliferation, and increased osteoblast maturation. Apoptosis now accompanies fusion, but is restricted to bone fronts coming into contact. During the process of suture fusion, we show that the progress of differentiation is accelerated, which correlates with the increased differentiation of mutant cells in vitro. Our studies suggest that the major determinant of Fgfr2-induced craniosynostosis is the failure to respond to signals that would halt the recruitment or the advancement of osteoprogenitor cells at the sites where sutures should normally form. The process of coronal suture fusion in this Apert syndrome mouse model resembles that in human Apert patients, suggesting the suitability of this model for developing effective therapies for the human condition.

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EFFECT OF CHRONIC POSTNATAL EXPOSURE TO HYPOXIC ENVIRONMENT ON CANAL NETWORK FORMATION IN INFANT RAT CORTICAL BONE

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Background: Chronic hypoxia retards skeletal growth. However, the morphological response of cortical bone microstructure (i.e., vascular canal network) to hypoxia is unknown. Using monochromatic synchrotron radiation CT, we investigated the structure of vascular canal network in infant rats exposed to chronic hypoxia.

Materials and Methods: Tibiae were harvested from 5- and 9-week-old male Wistar rats (hyp-5 and -9, n=8 each) housed in a hypoxic chamber (12-14%O2) after birth and from 4- and 5-week-old rats (cnt-4 and -5, n=8 each) maintained in ambient air. No difference was found in body weight and tibia length between hyp-5 and cnt-4 and between hyp-9 and cnt-5. The 2.5-mm-long diaphysis immediately proximal to the tibio-fibula junction was imaged with 20-keV X-ray energy and a voxel size of 3.1 μm at SPring-8 (Harima, Japan). The canal network was segmented by simple thresholding at a bone mineral density of 0.8 g/cm3 and the following indexes were computed: canal volume fraction (CaV/TV), mean density of canals penetrating transverse sections (CaN/TA), canal diameter (CaD), density of canal links (CaLn/TV), and densities of canal ends in the endocortical surface (CaN/ES) and the periosteal surface (CaN/PS).

Results: Bone and medullary volumes were similar in hyp-5 and cnt-4 and in hyp-9 and cnt-5. However, the canal network structure differed between hypoxic and control groups. In hyp-5, CaLn/TV was higher and CaN/ES and CaN/PS were lower than in cnt-4 (349±115 vs. 217±97 mm-3, 32±5 vs. 42±7 mm-2, and 28±4 vs. 37±7 mm-2, respectively) although CaV/TV, CaN/TA, and CaD did not differ between hyp-5 and cnt-4. Lower CaN/ES and CaN/PS in hyp-5 indicate less prominent invasion of microvessels into cortical bone, probably being unfavorable for trans-cortical perfusion. On the other hand, both hyp-9 and cnt-5 showed similar CaN/TA, CaLn/TV, CaN/ES, and

CaN/PS, but CaV/TV and CaD were lower in hyp-9 (2.7±0.5 vs. 4.5±1.0% and 15.0±0.5 vs. 16.6±0.8 μm, respectively), implying higher vascular resistance than in cnt-5.

Conclusions: Chronic hypoxia changes the structure of vascular canal network in infant cortical bone, probably leading to a reduction in bone perfusion. Thus further reduction in O2 supply to bone will occur, thereby contributing to skeletal growth retardation.

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MYOCYTE ENHANCER FACTOR 2C IS REQUIRED FOR PROPER MC3T3-E1 OSTEOBLAST DIFFERENTIATION

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Myocyte Enhancer Factor 2c (Mef2c) is a MADS-box transcription factor required for muscle cell development. More recently, Mef2c has been demonstrated to participate in skeletal development by regulating chondrocyte differentiation. Additionally, its role in bone formation was further defined by tissue-specific inactivation in cells of the neural crest, which resulted in severe craniofacial defects. We identified Mef2c as a potential regulator of osteoblast differentiation through microarray gene expression analysis which revealed Mef2c transcript levels were significantly elevated in differentiating MC3T3-E1 cells (10.2 fold, $p = 0.001$). The levels of Mef2c expression was investigated through out an osteoblast differentiation time course. Mef2c displayed two distinct peaks in expression occurring at days 7 (22 fold) and 16 (16 fold) coinciding with the onset of ECM maturation and active mineralization phases respectively. The role of Mef2c in MC3T3-E1 differentiation was investigated via overexpression and knockdown studies. The overexpression of Mef2c resulted in the significant augmentation of alkaline phosphatase activity and accordingly, the osteoblast phenotypic genes bone sialoprotein (BSP) and osteocalcin (OSC) were also significantly elevated. Short hairpin (sh) RNA mediated knockdown of Mef2c transcripts resulted in significantly reduced alkaline phosphatase activity and decreased ECM mineralization. Consistently, significant decreases in BSP and OSC transcript levels were observed. The steady increase in Mef2c transcript levels observed during the initial phase of MC3T3-E1 osteoblast differentiation suggested a possible role for Mef2c in regulating cellular proliferation. To address this possibility, an MTT assay was implemented to investigate the effect of shRNA mediated knockdown of Mef2c on cellular proliferation. The assay revealed that cell numbers were significantly decreased as a consequence of Mef2c gene silencing suggesting the transcription factor participates in osteoblast proliferation. In conclusion, Mef2c gene expression is induced during the differentiation of MC3T3-E1 osteoblasts and is necessary for proper matrix mineralization.

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PERSONAL ULTRAVIOLET EXPOSURE AND VITAMIN D SYNTHESIS

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It is currently assumed that incidental UV exposure, particularly in a sunny climate should provide adequate vitamin D status for the population. This research was undertaken to test this assumption among healthy free-living adults aged 18 to 87 years, in southeast Queensland, Australia (27°S), during late February/early March 2007 (Australian summer). 42 adults (40 males, mean age 42±21 years) participated in this project, by having a blood sample taken to assess baseline serum 25(OH)D status and answering a self-reported questionnaire which sought demographic data and information about sun exposure. Participants were distributed UV dosimeters to assess personal sun exposure. 48 hours post baseline blood sample, a second sample was collected along with collection of the used UV dosimeters. The mean blood serum 25(OH)D at baseline was 70nm/L (s.d. 1.6 nm/L) and 48 hours post baseline was 78nm/L (s.d. 1.5nm/L). The median personal UV exposure in the final 24 hours the median personal UV exposure was 8.2 MED with a range of 0.2 MED to 7.4 MED. No significant correlation was found between personal UV exposure over the 48 hour period and change in 25(OH)D status between baseline and final collection. No relationships were also found between sunscreen use, age or gender. These results suggest that short term sun exposure does not impact on the 25(OH)D status, even though high exposures were of such intensity to promote the production of 25(OH)D.

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THE MC3T3E1 SUBCLONE 4 CELL-LINE AS AN IN VITRO MODEL IN THE STUDY OF THE ANABOLIC ACTION OF PARATHYROID HORMONE (PTH)

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The ability to recapitulate a PTH anabolic effect in cell culture has been a long desired system. Published studies of PTH increasing the mineralisation of extracellular matrix of osteoblastic cells in long-term culture have at times been difficult to reproduce or have required complex, intermittent treatment protocols, or both. More recently, Rey et al. (Bone, 2007) reported that an anabolic response could be obtained with continuous PTH (1-34) treatment of subclone 4 of the MC3T3-E1 cells (MC4 cells), with an increase in mineralisation by approximately 5-fold compared to untreated controls. We examined the effect of PTH 1-34 on mineralisation, ALP activity and ALP mRNA expression in the MC4 cell-line in our laboratory. MC4 cells were cultured in α-MEM and 10% FCS for 8 days, and from day 8 were treated continuously with 1nM, 10nM or 100nM PTH 1-34 and 10mM of β-glycerophosphate for up to 3 weeks. In contrast to the findings of Rey et al., a time course evaluating the effect of PTH 1-34 on mineralisation in the MC4 subclone demonstrated dose-dependent inhibition of matrix mineralisation with PTH treatments ($p<0.01$). To examine the effect of PTH on alkaline phosphatase activity and ALP mRNA, MC4 cells were cultured for 8 days then treated with 10-7 M PTH for 24 hours. PTH 1-34 increased alkaline phosphatase activity 9-fold ($p<0.05$) at 24 hours, in keeping with published results. A 10-fold increase in ALP mRNA expression was seen in MC4 cells treated with PTH 1-34 for 24 hours ($p<0.05$). Although cell culture is an invaluable tool in the study of bone biology, it has limitations in its capacity to reproduce the complexities of the in vivo system. Furthermore, reproducing in vitro conditions can be difficult, with the results of PTH treatment in MC4 cells in our laboratory resulting in inhibition of mineralisation in contrast to published data, and is consistent with findings in other stromal cell lines. Whilst all culture conditions were as described by Rey et al., we cannot exclude variations in fetal calf serum as a possible contributing factor for the different results obtained with this cell-line.

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COMPARISON OF 25-HYDROXY-VITAMIN D DETERMINATION THROUGH A COMMERCIAL PATHOLOGY LABORATORY AND A RESEARCH LABORATORY

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Vitamin D plays a major role in the regulation of calcium and phosphorus absorption from the small intestine and is therefore vital for skeletal development. Thus, vitamin D deficiency can, for example, lead to an increased risk of osteoporosis and bone fracture. Furthermore, low levels of vitamin D have been linked to decreased muscle strength as well as increased risk of colon, prostate and breast cancers.

Vitamin D is produced when 7-dehydrocholesterol, located in the epidermis of the skin, is irradiated with UV. After entering the bloodstream and being transported into the liver, vitamin D is hydroxylated to 25-hydroxy-vitamin D, which is the main circulating metabolite. A further hydroxylation reaction in the kidneys leads to the formation of 1,25-dihydroxy-vitamin D. This is the active steroid hormone which regulates calcium homeostasis.

Vitamin D is efficiently removed from the bloodstream through the first hydroxylation reaction in the liver, which happens within several hours after the release of the vitamin D from the skin, and is therefore no longer detectable. The second hydroxylation reaction that forms 1,25-dihydroxy-vitamin D is tightly regulated and the concentration of this metabolite does not necessarily reflect an individual's vitamin D status. Therefore, in order to gain meaningful information about a person's vitamin D status, the concentration of 25-hydroxy-vitamin D needs to be measured.

Determination of 25-hydroxy-vitamin D is being offered from commercial pathology laboratories. We compared the results of 40 samples analysed in a commercial laboratory with our own research laboratory's measurements. Both methods used the same chemiluminescent immunoassay technology; however, in our laboratory we applied several QC/QA methods: internal quality controls were included in every batch of analysed samples and external quality was assessed by participating in DEQAS, the Vitamin D External Quality Assessment Scheme. Additionally, all samples were analysed by high-pressure liquid chromatography, performed in our laboratory. The results from these three methods were compared using different statistical methods and these analyses will be discussed, together with advantages and disadvantages of the methods.

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BONE CELL AUTONOMOUS EFFECTS OF OSTEOACTIVIN/GPNMB IN VIVO AND EX VIVO

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Osteoactivin/Glycoprotein nmb (OA/gpnmb) is a transmembrane glycoprotein. The protein is synthesized, processed and heavily glycosylated by osteoblasts. Its expression is associated with increased osteoblast differentiation and matrix mineralization. We have previously shown that OA/gpnmb expression in osteoblasts is regulated by BMP-2 through the Smad-1 signaling pathway. In this study, we used a mouse model with a naturally occurring mutation in the OA/gpnmb gene resulting from a premature stop codon that leads to the production of a truncated OA/gpnmb protein with no biological functions. OA/gpnmb mutant mice develop osteoporosis with age when compared to normal, wild type (WT) littermates. Histological and micro-CT measurements of femurs in mutant mice revealed a decrease in bone volume (BV/TV), trabecular number (Tb.N), and trabecular thickness in OA/gpnmb mutants compared to WT controls. Primary osteoblasts were generated from newborn OA/gpnmb and WT mice and examined for their differentiation ex vivo. All markers for early (alkaline phosphatase activity and collagen type I expression) and late (nodule formation, matrix mineralization and osteocalcin production) osteoblast differentiation were significantly reduced in the OA/gpnmb mutant osteoblasts compared to controls. We also examined bone marrow stromal cells isolated from OA/gpnmb and WT mice and testing their ability to differentiate into osteoblasts. Colony forming unit-fibroblasts (CFU-F) and CFU-osteoblasts (OB) (determined by alkaline phosphatase staining) were significantly reduced in mutant compared to WT mice. These data suggest that OA acts as positive regulator of osteoblast differentiation and function in vivo. We next examined osteoclast differentiation using a co-culture system established using normal osteoblasts as feeder cells and bone marrow (monocyte/macrophage) obtained from either OA/gpnmb mutant or WT mice in the presence of 1,25(OH)2 vitamin D3 and PGE2. Osteoclast formation/differentiation was determined by TRAP-staining and actin ring formation. Co-culture of bone marrow cells isolated from OA/gpnmb mutant mice and WT osteoclasts showed marked increase in osteoclast numbers and size when compared to osteoclasts generated from normal bone marrow cells and normal osteoblasts. These data suggest the OA/gpnmb acts as a negative regulator of osteoclast formation in vivo. Collectively, these data suggest that OA/gpnmb acts to regulate bone remodeling by positively affecting osteoblastogenesis and negatively regulating osteoclastogenesis in vivo.

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ANALYSIS OF BONE IN POMC KNOCKOUT MICE

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The proopiomelanocortin (POMC) gene encodes numerous peptide hormones secreted by the CNS, the pituitary, and other tissues in the periphery. These hormones include α-, β- and γ-melanocyte stimulating hormones (MSH), adrenocorticotropin (ACTH), β-lipotrophin, and β-endorphin. Roles for these hormones have been demonstrated in pigmentation, body weight and metabolism regulation, steroid hormone production, and pain modulation. In the hypothalamus and in the peripheral circulation, α-MSH is secreted in response to elevated leptin levels.

Several types of bone cells express subsets of the melanocortin receptors as well as the ACTH receptor. In vitro , α-MSH has been shown to increase bone turnover, increasing both osteoblast proliferation and osteoclastogenesis, while systemic administration of α-MSH reduces bone volume in vivo. There are few recent studies of the direct effects of ACTH on bone cells, and its activities in vivo are often confounded by the numerous steroid hormones it stimulates. β-endorphin is one of several endogenous opioids and this family has generally been shown to be anabolic to bone.

POMC knockout mice have non-functional adrenal glands with reduction or loss of all adrenal hormones, show increased linear growth, are morbidly obese and develop pituitary tumors with age.

In this pilot study, we examined tibia from POMC null mutants for changes in their bone characteristics before the onset of obesity (aged 8-10 weeks, 3 females per group) using computer assisted microtomography. Cortical thickness was significantly increased in POMC null mice (0.18mm±0.009SEM vs. 0.13±0.003, p=0.0139) versus controls.

Changes in trabecular bone in POMC null mice did not reach significance in several measurements: trabecular thickness (0.046mm±0.002 vs. 0.044mm±0.001), trabecular separation (0.20mm±0.006 vs. 0.23mm±0.03) or bone surface (12.1mm2±2.1 vs. 10.83mm2±0.37). Average femur length (13.9mm±0.15 vs. 13.4mm±0.23) and growth plate thickness also did not reach significance in this small number of animals, although interesting trends were again seen in the POMC null animals.

This preliminary study shows that ablation of POMC signaling results in changes in bone morphology consistent with some but not all of the constituent POMC hormones. We suggest that the combined loss of α-MSH and reduction of steroid hormone signaling may be responsible for increasing the rate of osteoblast proliferation and/or reducing the rate of osteoclast formation or function in bones, potentially leading to increased linear length. Changes in POMC hormone signaling impact bone formation during mammalian development and warrants further investigation for possible links to central control of bone metabolism.

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THE CHARACTERISATION OF THREE TCF-LUCIFERASE CONSTRUCTS IN PTH AND WNT INDUCED CANONICAL PATHWAY SIGNALLING

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The observation that Wnt signalling was critical in bone metabolism has been a major development in the area of bone biology. However, many questions regarding Wnt signalling in the regulation of osteoblast differentiation remain unanswered, including the complex interactions between PTH and Wnt signalling pathways. The study of Wnt action has resulted the development of TCF-reporter plasmids capable of monitoring canonical signalling in vitro. We have characterised and determined the utility of different TCF-constructs and their negative controls as they pertain to lithium chloride, Wnt 3a and PTH induced changes TCF/LEF-responsive gene transcription in osteoblastic cells. Three different TCF-constructs were used, and these differ in the number of TCF target sequences or the minimal promoters driving expression of a luciferase gene: TOPflash contains one set of three copies of the TCF binding site upstream of a c-fos minimal promoter; Upstate Biotech TOPflash (UB TOPflash) contains two sets of three copies of the TCF binding site upstream of a Thymidine Kinase minimal promoter; 8xTOPflash has 8 TCF binding sites with a minimal TA viral promoter. UMR 106.01 were transfected with the TCF-constructs and treated with LiCl, Wnt 3a and PTH. Treatment with 40mM LiCl resulted in a 10-fold increase in TOPflash, a 700-fold increase in UB TOPflash and a 300-fold increase in 8xTOPflash luciferase response. When monitoring responses to Wnt 3a, 8xTOPflash was the most sensitive TCF-vector demonstrating an increase in luciferase response with concentrations greater than 10ng/ml and a 400-fold increase with Wnt 3a concentrations of 100ng/ml. In contrast, TOPflash was stimulated 2-3 fold by 100ng/ml of Wnt 3a and UB TOPflash was stimulated 8-fold by 100ng/ml of Wnt 3a. When comparing the response of the TCF-reporters to PTH and PTHrP, N-terminal PTH/PTHrP increased TOPflash and UB TOPflash luciferase activity in a dose-dependent manner, whilst 8xTOPflash was not responsive to PTH/PTHrP. The various TCF-reporter constructs varied greatly in their responsiveness to LiCl and Wnt 3a, but the absent response of 8xTOPflash to PTH/PTHrP raised questions regarding the true biological effect of PTH on Wnt signalling. Recent evidence however supports PTH activation of ß-catenin signalling in osteoblasts in vitro and in vivo.

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ODANACATIB INCREASES BONE STRENGTH AND MAINTAINS BONE QUALITY IN ESTROGEN DEFICIENT ADULT RHESUS MONKEYS

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Odanacatib (ODN), a selective inhibitor of Cathepsin K (CatK) is a promising agent to treat osteoporosis. We examined bone strength/quality in the adult skeleton. Ovariectomized (OVX) rhesus monkeys (13-19yrs, N=8-11) were given ODN (0, 6, or 30 mg/kg (qd, PO)) for 21 months. BMD was measured quarterly. Central femur (CF) was tested in three-point bending, femoral neck (FN) in shearing, and vertebral body (LV4) in compression. Load-deformation curves were used to calculate Ultimate Load (F.U), Stiffness, Toughness, and Ductility. Peripheral quantitative computed tomography was used to determine BMC and cortical thickness (CFCTh) at the site of failure. Baseline LVBMD differed little among groups. Final LVBMD was 11% and 17% higher; HBMD was 10% and 16% higher;

and FNBMD in ODN treated animals was 11% and 12% higher than OVX+0. CFCTh was higher (both 16%), as were CFF.U (25-32%) and CFStiffness (23-33%) with ODN than OVX+0. FNF.U was 17-19% higher and LV4F.U was 18% higher with ODN. Toughness and ductility (LV4) did not differ among groups. CFF.U and CFStiffness correlated to CFBMC (r=0.95; P<0.001 and r=0.82; P<0.001; N=28). LV4F.U and LV4Stiffness correlated to LV4BMC (r=0.79; P<0.001 and r=0.46; P<.02; N=27). CFF.U and CF Stiffness correlated with CFCTh (r=0.70; P<0.001 and r=0.46; P<0.02; N=28). ODN-treated monkeys have higher bone mass in sites of human osteoporotic fracture, greater bone strength and stiffness in the central femur, and trends toward better bone strength at the femoral neck and spine. The relationship of bone mass to bone strength is normal in cortical and trabecular bone. ODN increases bone mass and strength, while maintaining normal bone quality in adult rhesus monkeys.

	OVX+0	OVX+6mg/kg ODN	OVX+30mg/kg ODN
CFCTh (mm)	1.82±0.26	2.10±0.14*	2.12±0.226**
CFF.U (N)	1203±195	1507±251**	1589±203***
CF Stiffness (N/mm)	875±127	1067±248*	1157±212***
FNF.U (N)	2026±444	2405±307	2379±467
L4F.U (N)	3398±797	4017±1012	4017±972
L4 Ductility (mm)	0.979±0.207	0.990±0.144	0.942±0.170

Mean±SD; *(P<0.05); **(P<0.01); ***(P<0.001) vs. OVX+0
(H)-hip, (LV)-spine, CF-central femur, FN-femoral neck

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MUSCLE CONTRIBUTIONS TO THE HIP JOINT CONTACT FORCE IN NORMAL WALKING

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Hip fracture due to osteoporosis is a significant public health problem. Hip fractures can result from an impact with the ground, or they can occur during the performance of daily activities such as standing up from a chair or during gait. Identifying the loading conditions under which the proximal femur is likely to fail would improve our understanding of the biomechanical causes of hip fracture and may aid in the development of more targeted muscle-strengthening exercises for preventing these injuries. The overall goal of our on-going work is to develop non-invasive methods for accurately assessing hip fracture risk in individual elderly persons. The specific aim of this study was to describe and explain individual muscle contributions to hip-joint loading during normal gait.

A three-dimensional dynamic simulation of walking was used to examine the contributions of the lower-limb muscles to the hip contact force. The body was modeled as a 10-segment, 23-degree-of-freedom articulated linkage actuated by 54 Hill-type musculotendon actuators, with foot-ground interaction modeled using stiff springs distributed under each foot. The simulation of walking was obtained by solving a dynamic optimization problem for the muscle excitations that minimized the metabolic energy expenditure per unit distance walked.

Not surprisingly, the model simulation results showed that the muscles which contributed most to hip contact force were those that span the hip. Muscles that span the back, knee and ankle joints also contributed, but with much less influence. The muscles that contributed most to hip contact force were the two hip extensors, gluteus maximus and hamstrings, and the hip abductor, gluteus medius. We also perturbed muscle strength in the model to study the effect on hip-joint contact force. A modest reduction in gluteus medius strength led to a considerable increase in hip contact force. This appears to be directly attributed to increases in the compensating muscles of rectus femoris, hamstrings, and tensor fasciae latae. Other mild weakness conditions (of hip flexors, quadriceps, and hip extensors) showed no noticeable change in contact force. Further studies could evaluate the effect of hip muscle strength on the distribution of stresses and strains along the femoral neck.

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DETRIMENTAL BONE EFFECTS OF SMOKING AND A HIGH FAT DIET IN MICE

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Smoking, high-fat diet and obesity are important risk factors for many globally-prevalent diseases. While cigarette smoking has been shown to be adverse to bone density and fracture risk, greater body weight may be protective against

fractures. The aim of this study was to examine the effect of cigarette smoking and a high fat diet (HFD) on bone mass, density, and estimated bone strength.

After acclimatization, 40 5 week-old male b alb/c mice were randomly divided into four equal groups with similar average body weight for 7 weeks intervention: sham fed chow (sham/chow), sham fed high-fat diet (sham/HFD), smoke exposure (2 cigarettes, twice/day, 6 days/week) fed chow (SE/chow), and SE fed HFD (SE/HFD). Bone densitometric and geometric parameters of the tibia were measured using pQCT.

Sham/HFD mice were heavier than chow-fed and SE groups after the intervention ($p < .001$). SE/chow mice were lightest. Bone results were adjusted for body weight to account for differences between groups. Cortical bone measures were obtained at the tibial shaft (50%). Control mice (sham/chow) had the greatest cortical content, cortical thickness and periosteal circumference. Compared with control mice (sham/chow), smoking alone (SE/chow) resulted in a more slender bone; SE/HFD preserved periosteal circumference but with a thinned cortex due to endosteal expansion; and HFD alone (sham/HFD) resulted in mild reductions in cortical content, thickness and periosteal circumference. Tibial length and bending strength were similar between groups, although SE/chow mice had lower y-axis bending strength possibly due to smaller periosteal circumference and cortical thickness. Trabecular bone measures at the 5% site did not differ between groups.

Site	Sham/Chow	Sham/HFD	Smoke/Chow	Smoke/HFD	p Value
Weight (g)	26.36 (1.26)	28.69 (2.64)	22.62 (1.33)	25.34 (1.28)	<0.001
Cortical Content (mg/mm)	0.905 (0.09)	0.860 (0.12)	0.831 (0.05)	0.795 (0.05)	0.047
Cortical Thickness (mm)	0.253 (0.02)	0.240 (0.02)	0.237 (0.01)	0.220 (0.01)	0.001
Endosteal Circumference (mm)	2.220 (0.09)	2.199 (0.09)	2.162 (0.10)	2.403 (0.27)	0.006
Periosteal Circumference (mm)	3.815 (0.13)	3.709 (0.19)	3.655 (0.12)	3.789 (0.25)	0.188
Bending Strength x-axis (mm*3)	0.121 (0.03)	0.110 (0.02)	0.116 (0.01)	0.110 (0.01)	0.597
Bending Strength y-axis (mm*3)	0.122 (0.03)	0.123 (0.04)	0.106 (0.02)	0.116 (0.03)	0.556

Results - Mean (SD), p Value for between group analysis (oneway ANOVA)

In this novel model of cigarette exposure, smoking was adverse for cortical bone, particularly cortical content, thickness and periosteal circumference resulting in a thinner bone that is likely to be fracture-prone. Smoking interacted with a high fat diet and the combination resulted in cortical thinning with preservation of periosteal circumference. This may also increase bone fragility.

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SHORT-TERM VS LONG-TERM DURATION OF AED (ANTI-EPILEPTIC DRUG) PHARMACOTHERAPY: EFFECTS ON BONE HEALTH PARAMETERS

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Anti-epileptic drug (AED) therapy is a major iatrogenic cause of bone loss and fracture.

Evidence suggests an association between duration of AED-treatment and the risk of bone disease. However, the characteristics of this relationship and the influence of other factors have not been fully elucidated.

We investigated the role of the duration of AED therapy and its impact on bone by assessing the difference in bone health parameters in two AED-treated populations, comparing newly-diagnosed epileptic patients taking AEDs for ≤6 months and longer-term AED-taking patients (>6 months therapy) using a cross-sectional study design. Mean differences between groups in dual energy xray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) parameters were evaluated and the impact of several putative risk factors (age at AED commencement, gender, AED-type, polytherapy and dosage) was explored. The study sample consisted of 91 participants. Population 1 comprised 34 newly-diagnosed, short-term AED-taking patients (56% male, 44% female) with a mean age (±SD) of 42.08 ±14.0 y. Population 2 consisted of 58 longer-term, AED-taking patients (59% male, 41% female) with a mean age of 44.29 ± 17.32 y. Of the 91 participants, pQCT scans were available in 62. Data were normally distributed and adjusted for age, height and weight. Parametric independent t-tests were then utilised to assess population mean differences.

Total hip areal bone mineral density (aBMD) presented a highly-significant difference, with those on longer-term AEDs exhibiting lower values (0.97 ± 0.014 g/cm2,mean ±SEM) than short-term users (1.05 ± 0.018), p=0.002 (two

tailed). Total body aBMD was marginally different between short-term (1.14 ± 0.018 g/cm2) and long-term users (1.10 ± 0.013), p=0.051 (two tailed).

pQCT parameters were assessed at the 4% and 38% non-dominant radial and tibial bone sites. The 4% tibial trabecular density was the only parameter to display a highly-significant difference between groups, with long-term users (244.36 ± 5.55 mg/cm3) exhibiting lower values than their short-term counterparts (583.71 ± 11.53), p<0.01 (two tailed).

Preliminary examination of clinically-relevant sub-groups revealed several significant mean differences; however, a larger sample is required to confirm these findings. Observations to date provide evidence of poorer bone health with increased AED therapy duration.

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OSTEOPOROSIS AND VIT B12 DEFICIENCY AMONG METFORMIN USERS

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Human skeleton is one of the largest organ systems in the body receiving about 10% of the cardiac output and bone is a dynamic tissue, remodeled continuously during whole life through bone remodeling units (BRUs).Deficiencies of Vit. D and Vit. K are well known for pathophysiology of metabolic bone disease including osteoporosis. Vit A toxicity has also been implicated clearly for bone metabolism disorders.However,role of Vit B12(cobalamin) is not very clear for etiology of osteoporosis except occasional report of suggesting its deficiency in osteoporosois.Vit B12 deficiency is common among vegetarians and its a major concern for them.Megaloblstic anemia and neuropsychiatric manifestations are commonly seen due to Vit B12 deficiency. Chronic use of metformin has been reported as one of the causes of Vit B12 deficiency. Here we report three cases of osteoporosis (spine and hips) among patients who have also been detected as Vit B12 deficient with chronic metformin usage. All the three adult males aged 49,54,&56 yrs were on metformin for the past two years for treatment of T2DM.Detailed clinical history including various drugs intake affecting the bone mineral metabolism and systemic examinations were recorded.These cases were eugonadal and euthyroid.They were investigated and complete haemogram including peripheral blood smear, serum electrophoresis, liver function tests, renal function tests,glycemic status along with RBC folate levels, vit B12 levels, vit D status(25-hydroxy Vit D),calcium,phosphorus, PTH, osteocalcin, beta crosslaps, total P1NP,BMD (DEXA)of hips, spine and wrist were estimated.BMD (T Score) of the patients at spine and hips were:caseI -2.6 &-1.8, caseII -2.8 & -2.2, caseIII-3.2 & -1.6 and levels of Vit B12 (Chemiluminescence) were 134, 164, 87 pg/ml respectively(Vit B12 ref values 211.0-911.0 pg/ml).25-hydroxy Vit D and PTH levels were normal in all three cases.Observation of metformin induced Vit B12 deficiency in present cases highlights the importance of complex process of osteoporosis and its correlation with Vit B12 deficiency. We suggest that more clinical studies need to be done to explain the cause and effect relationship of metformin usage, B12 deficiency and osteoporosis.

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LOW DIETARY CALCIUM INTAKE THROUGH MIDDLE SCHOOL GRADES AND ITS CORRELATES IN A DIVERSE SUBURBAN US COMMUNITY

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BACKGROUND: Adequate calcium and vitamin D intake is crucial during adolescence to attain peak bone mass. There is data on inadequate consumption of these nutrients in different population but predictors for suboptimal intake are varied and are not well defined.

OBJECTIVE: To evaluate the effect of gender, lifestyle factors, taste preferences, personal health beliefs and meal patterns in healthy middle school students in New York to identify the students' stereotypes and misconceptions.

DESIGN: A total of 250 students aged 10-14 years were selected from a public school in suburban New York and information was collected on calcium and vitamin D intake through a semi-quantitative food frequency questionnaire. A 15–question survey was developed utilizing standard validated questionnaire and reviewed by the Middle School

Director of Science Curriculum to assess areas of knowledge on Vitamin D, Calcium nutrition and bone health. Each item was a multiple choice question with one correct response.

RESULTS: A moderate level of knowledge on bone health among students was demonstrated; with high scores on knowledge of the effects of the calcium on bones, and knowledge of osteoporosis and bone development. Girls were clearly more knowledgeable than boys. About 90% of students reported inadequate consumption of milk. Average reported intake of milk was only 1.38 cups/day (less than 500 mg calcium daily). Calcium supplements were used by 41 % students. 57% students reported not taking part in any physical exercise daily. The commonest misconception was that the bones are not living organs [37%]. Only 31 % knew that bones grow significantly during middle school. The most common reasons for avoiding milk were poor taste [82%], lactose intolerance or restricted dietary habits.

CONCLUSION: Attitude change lags behind knowledge. Future school calcium interventions need to take into account students' attitudes and perspectives. They should aim at motivating attitude change and preventive behavior through consistent and repeated calcium education messages that are supported by a calcium conscious school environment. Our findings have important implications regarding institution of dietary health strategies to promote skeletal health among adolescents in our community.

(1) Surgeon General Report on Bone Health: Prevention is Key. Press release at http://www.hhs.gov/news/press/2004pres/20041014.html

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NORMAL-CALCIUM, LOW-PROTEIN, LOW-SALT DIET FOR THE CONTROL OF RECURRENT STONES IN IDIOPATHIC HYPERCALCIURIA IN WOMEN

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Idiopathic hypercalciuria (IHC) is a very common condition and the main cause of nephrolithiasis. The traditional low-calcium diet is often ineffective in preventing the recurrence of stones, and may cause bone loss.

We studied the effects of a normal-calcium, low-protein, low-salt dietary intake in 38 pre-menopausal women (39.8±7.4 years) with a history of calcium oxalate stones and IHC (at baseline: average calcium intake 480±90 mg/day; water intake 1.8±0.3 L/day). IHC was diagnosed upon the finding of urinary calcium excretion >250 mg/day in at least 3 different urine samples, after excluding other known causes of hypercalciuria.

The 38 women were followed up for 4 years and we collected also their clinical records of the 3 preceding years for comparison. At baseline, all women had a marked increase in urinary calcium (402±71 mg/day), and the strontium test showed increased intestinal calcium absorption in 21 of them (55.3%).

All women received detailed written information on a diet providing normal daily intake of calcium (1000 mg) and low intake of protein (70 g, mainly vegetal), salt (2.3 g), and oxalate-rich foods. They were also instructed to avoid the simultaneous intake of calcium and oxalate and to maintain a water intake of at least 2 liters/day.

Recurrent stones were observed in 6 (15.8%) women during the 4 years of follow-up, while in the preceding 3 years, stones had recurred in 17 (44.7%) women. The relative risk of stone recurrence with the new dietary regimen was 0.35 (95% CI 0.16 to 0.78, $p<0.05$).

At the end of the 4-year follow-up, urinary calcium was significantly decreased (223±59 mg/day, $p <0.01$), as well as urinary oxalate and urea.

Osteopenia, according to the WHO criteria, was present in 30 (78.9%) of patients at baseline (Z-score -1.7±0.3), and was unchanged at the end of follow-up (Z-score -1.5±0.2).

We conclude that a low-calcium diet to prevent calcium oxalate stones is not recommended in pre-menopausal women with IHC. A normal-calcium, low-salt, low-protein diet can reduce calcium excretion and the recurrence of renal stones, and will also be useful in preventing bone loss.

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MECHANISMS OF EROSIVE GOUT: MONOSODIUM URATE MONOHYDRATE CRYSTALS REDUCE OSTEOBLAST VIABILITY

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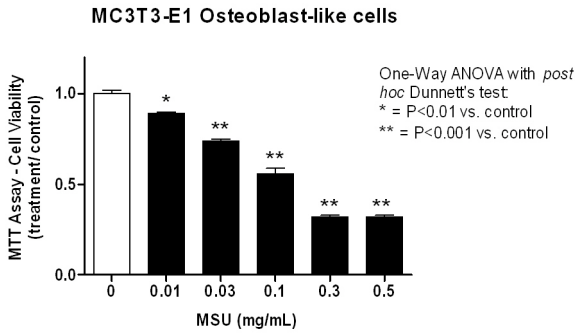
Gout is an inflammatory arthritis that is triggered by monosodium urate monohydrate (MSU) crystals within the joint. MSU crystals interact with surrounding cells and tissue within the joint causing inflammation. Bone erosion is a frequent manifestation of chronic gout, and leads to joint damage and deformity, with subsequent disability. We have recently shown that patients with erosive gout have disordered osteoclastogenesis and that MSU crystals indirectly promote osteoclast formation through interactions with stromal cells.

In this study we investigated the effect of MSU crystals on the proliferation of bone forming osteoblast cells. MSU crystals were prepared by recrystallisation of uric acid and added to MC3T3-E1 osteoblast-like and primary rat osteoblast cell cultures.

Cell proliferation was assessed following MSU treatment. MSU crystals decreased both MC3T3-E1 osteoblast-like and primary rat osteoblast cell viability in a dose-dependent manner. This inhibitory effect was maximal at the higher concentrations of MSU, 0.3 and 0.5mg/mL.

These results indicate that MSU crystals may lead to the development of bone erosion in gout both through promotion of osteoclast formation and reduction of osteoblast viability.



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OSTEOACTIVIN IN ALVEOLAR BONE REGENERATION IN EXTRACTION SOCKETS IN NORMAL AND DIABETIC RAT

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Delayed wound healing and impaired alveolar bone regeneration is associated with the metabolic abnormalities characteristic of poorly controlled diabetes mellitus. Osteoactivin (OA) is a novel bone anabolic factor that is known to play a critical role in osteoblasts differentiation and function. OA has been shown to be highly expressed at sites of active osteogenesis in vivo. In this study, we investigated the expression and localization of OA during healing and bone regeneration in teeth extraction sockets in normal and streptozotocin-induced diabetic rats. One week after induction of diabetes using Streptozotocin intraperitoneal injections, rats were examined for body weight, glucoseuria and glycosemia to confirm the diabetic condition during the study. Rats underwent extraction of first and second right maxillary molars. Animals were sacrificed at three, five, seven and ten days post-extraction. OA localization was detected by immunohistochemistry staining. Expression of OA and other bone-related genes were determined by RT-qPCR analysis. An in vitro study was undertaken to support the results of the in vivo study. MC3T3-E1 osteoblasts like cells were isolated and cultured with different concentrations of D-glucose (15 and 25mM). Control cultures were treated with medium containing 5.5mM —glucose, standard glucose concentration in culture medium. Alkaline phosphates staining, calcium deposition and micro-array analysis of osteoblasts related genes were evaluated. Effect of OA peptide on both control and glucose treated cultures was determined by calcium deposition and expression of osteoblasts related genes (by RT-qPCR analysis). OA expression in differentiating osteoblasts in diabetic extraction

sockets was significantly decreased when compared to normal animals. Expression of OA and other bone-related genes determined by RT-qPCR analysis showed a significant reduction in sockets of diabetic animals compared to controls. In vitro study showed inhibition of osteoblast differentiation and extracellular matrix maturation induced by glucose treatment. In addition treatment with OA peptide seemed to rescue the effect of glucose determined by alkaline phosphates staining, calcium deposition and expression of osteoblasts related genes in both control and glucose treated cultures. Osteoactivin plays a role in bone regeneration and formation in teeth extraction sockets. Reduction of OA expression level in extraction sockets in diabetic rats may play a role in the delayed healing and impaired alveolar bone regeneration in these animals. Future studies will explore the mechanism by which glucose effect on OA expression as an extracellular matrix protein that promotes osteoblast-mediated bone formation.

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OSTEOCYTE LACUNAR DENSITY VARIATION IN THE MIDSHAFT FEMUR OF PEOPLE OF ANGLO-CELTIC AND BANTU ANCESTRY

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Mammalian osteocyte lacuna densities reflect the rate of osteoblast proliferation, transformation, and incorporation into bone as osteocytes during growth. While it is well researched that interspecific lacuna densities vary inversely with body size, little is known about within-species variability with body size.

OBJECTIVES: Our aim is to characterize osteocyte density variation around the cortex of human midshaft femur samples obtained from individuals of known life history from the Melbourne Femur Collection (MFC) derived from the Victorian Institute of Forensic Medicine, Melbourne, Australia, and the University of Malawi College of Medicine (UMCOM), Blantyre, Malawi.

METHODS: We performed real-time 3D circularly polarized light microscopy of 100-micrometer thick histological sections of secondary osteonal bone. Lacunae were visualized, counted, and extrapolated to numbers per cubic millimeter. Lacuna densities were determined from endosteal and periosteal locations from six MFC females, aged 38, 42, 49, 55, 62, and 88 and from three UMCOM females aged 28, 35, and 50 to date.

RESULTS (ages combined):

	MFC		UMCOM	
Sector:	Periosteal	Endosteal	Periosteal	Endosteal
Mean:	19614	22712	28008	23519

UMCOM osteocyte lacuna density is higher (avg. 26512, SD=4636) compared to that of the MFC (avg. 20444, SD=1426). When specific sectors are evaluated, there is no age-related decline in lateral periosteal sectors, whose lacuna densities, when linearly regressed against body mass (Least Squares Model), are described by a significant positive relationship ($r=0.90$, $p<0.02$); no statistical relationship between lacuna density and body height was found. By contrast, in our pilot study of UMCOM individuals, we found no relationship with body mass, but with body height it is negative and significant ($r=-1.00$, $p<0.01$; the strength of this relationship is a statistical coincidence and will surely moderate with larger sample sizes). This is much unexpected, and will need to be confirmed by further study and considered for its life history meaning.

CONCLUSION: Perhaps an explanation for relatively high osteocyte density in UMCOM compared to MFC individuals relates to a difference in the way mass and height are accrued in these two regional human populations. Improved assessments of lacuna density variation on larger samples by monochromatic synchrotron x-ray imaging are planned.

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DIFFERENTIAL EFFECTS OF ODANACATIB ON TRABECULAR VERSUS CORTICAL BONE FORMATION IN ESTROGEN-DEFICIENT RHESUS MONKEYS

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Odanacatib (ODN), a selective, reversible cathepsin K inhibitor, increases BMD and suppresses bone turnover in postmenopausal osteoporotic women. We demonstrated that ODN, at approximate clinical exposure, fully prevented BMD loss and maintained normal bone quality in newly ovariectomized (OVX) monkeys. Here, ODN effects on bone formation rate (BFR) in lumbar vertebrae (LV), proximal femur (PF), and femoral neck (FN) were further characterized. Rhesus monkeys (13-19 yrs) were assigned to four groups (n=8-11): intact, OVX + vehicle, and OVX + ODN (6 and 30mg/kg, q.d., p.o.). For histomorphometry, labels were given at 15-day intervals–calcein at 10 months and tetracycline prior to necropsy. Sections (parasagittal LV2, cross-sectional PF and FN) were evaluated. BFR was the distance between dual tetracycline labels, and long-term (LT)BFR was the distance between tetracycline and calcein labels. Surface-based mineralizing surface (MSBS), mineral apposition rate (MAR), and bone formation rate (BFRBS) were determined. Baseline LVBMD differed little among groups. 21-month ODN treatment resulted in gains of 10% and 18% LVBMD, 14% and 21% hip BMD, and 11% and 15% FNBMD in 6 and 30mg/kg ODN groups, respectively, vs. vehicle. ODN dose-dependently reduced LVBFR. LVBFRBS was unaffected with 6mg/kg, and reduced by 78% with 30mg/kg. Interestingly, ODN had no effect on endosteal BFR in PF and FN. Periosteal PFBFRBS was unaffected by 6mg/kg, and tended to be higher with 30mg/kg ODN. Periosteal FNBFRBS also tended to be higher with 30mg/kg ODN. Unlike bisphosphonates, ODN appeared to stimulate long-term periosteal bone formation vs. vehicle. LTMAR at the PF periosteum was 71% higher with 6mg/kg and was 3-fold higher ($p<0.005$) with 30mg/kg ODN. LTBFR at the PF periosteum was 2-fold higher with 6mg/kg and 5-fold higher ($p<0.003$) with 30mg/kg ODN. In FN periosteal bone formation, LTBFR was unaffected with 6mg/kg and 2-fold higher with 30mg/kg ODN. Taken together, ODN treatment at 6 and 30mg/kg prevented loss of bone mass in OVX-monkeys. Effects of ODN treatment on bone formation appeared to be bone-site specific. While dose-dependently decreasing bone formation in vertebral body trabecular bone, ODN treatment either left endosteal BFR unaffected or displayed long-term stimulation in hip periosteal bone formation.

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POTENTIAL PROTECTIVE ROLES OF METALLOTHIONEIN IN ACUTE METHOTREXATE CHEMOTHERAPY-INDUCED BONE DAMAGE

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Methotrexate (MTX), a dihydrofolate reductase inhibitor, is commonly used as a chemotherapeutic agent when administered in high doses. Although its usage has been implicated behind the observed bone growth defects seen in some childhood cancer survivors, much remains to be elucidated over the underlying cellular and molecular mechanisms. Metallothioneins (MT) are a family of zinc binding proteins and may act as intrinsic antioxidants and protect tissues from damage. Several studies have also shown that loss of MT expression enhances cellular susceptibility to apoptosis and increased oxidative stress. Here we investigated the role of MT-I and –II in a mouse model of MTX-induced bone damage. Male C57BL6 wildtype (WT) and MT-1/2 double knockout (KO) mice at 4-weeks old were injected subcutaneously with MTX (12.5 mg/kg) once daily for 3 consecutive days. Bone and growth plate specimens of tibia were collected on days 0, 5, 8 and 14 after MTX treatment. Histological analysis showed a decrease in total growth plate height with age across all groups. Interestingly, by day 14 after MTX treatment, the proliferative zone of the growth plate in WT mice was significantly reduced ($p<0.01$) compared to day 14 WT controls suggesting a reduction in endochondral bone formation. Furthermore, ex vivo culture of bone marrow samples showed a significant reduction in the number of alkaline phosphatase positive CFU-f colonies by day 14 of the WT MTX-treated group compared to the day 14 WT control group, suggesting a reduction of osteogenesis. To determine potential oxidative stress caused by MTX an in vitro glutathione enzymatic assay will be utilised. Future studies will involve testing effective zinc supplementation in protecting bone, via upregulation of MT in this model.

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CHONDROGENESIS USING MESENCHYMAL STEM CELLS WITH PCL-BASED SCAFFOLDS

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INTRODUCTION

In this study, we tested the in-vitro feasibility of PCL as a scaffold for MSC-based cartilage tissue engineering and the effects of scaffold modifications.

METHODS

Three modifications of porous PCL scaffolds were examined, i.e., 1) PCL / Pluronic F127, 2) PCL/collagen, and 3) PCL/Pluronic F127/collagen, in addition to 4) PCL-only. Circular scaffolds of dimension 7mm x 2 mm were prepared, and hMSCs at passage 3 cells were suspended in DMEM/F-12 medium at 5 x 105cell/20 μl . Scaffolds were then placed individually in the wells of a 48 well-plate. The scaffolds were left to stabilize inside the wells for 2 hours. Cell-scaffold composites were then placed in 15ml conical tubes, and cultured under DMEM/F-12 supplemented with 1% ITS, 10-7 M dexamethasone, 50 μM ascorbate-2-phosphate, 50 μM L-proline, 1 mM sodium pyruvate, and 5 ng/ml of TGF-β2 . Finally, after culturing for 21 days DNA levels were quantified, and qRT-PCR and GAG histological analyses were performed. The above procedure was repeated 5 times for each of the five donors.

RESULTS

The three surface-treated scaffolds had higher DNA contents than PCL-only scaffolds, and GAG contents in PCL/collagen and PCL/F127/collagen scaffolds were 1.5 and 1.2 fold higher than in PCL only scaffolds. Real-time PCR revealed that Col1A1 mRNA levels were lower in the three modified PCL scaffolds, and lowest in PCL/F127/collagen scaffolds. Sox-9 mRNA levels were elevated by 1.7-fold in PCL/collagen scaffolds and by 3.3-fold in PCL/F127/collagen scaffolds versus PCL-only scaffolds. Furthermore, COL2A1 mRNA levels were elevated by 4.7, 15, and 23-fold in PCL/F127, PCL/collagen and PCL/F127/collagen scaffolds, respectively, versus PCL-only scaffolds. On the other hand, Col10A1 mRNA levels were diminished in the modified PCL scaffolds, and were lowest in PCL/F127/collagen scaffolds. Histological findings generally concurred with GAG and RT- PCR findings, and demonstrated the affinity of PCL-based scaffolds for MSCs and the potentials of these scaffold in terms of inducing chondrogenic differentiation .

CONCLUSION

Our findings suggest that PCL-based porous scaffolds may be useful carriers for MSC transplantation in the cartilage tissue engineering field, and that collagen-based surface modifications further enhance the chondrogenic differentiation of MSCs.

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INCREASED VEGF IN THE SKELETON ENHANCES BETA-CATENIN ACTIVITY AND LEADS TO EXCESSIVE BONE DURING DEVELOPMENT, GROWTH AND ADULT BONE REMODELING

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Vascular endothelial growth factor (VEGF) and the Wnt pathway transcriptional regulator β-catenin both act broadly in embryogenesis and adulthood, including in the skeletal and vascular systems. Increased or deregulated activity of these molecules has been linked to cancer and bone-related pathologies. By using novel mouse models to locally increase VEGF levels in the skeleton, we found that embryonic over-expression of the major isoform VEGF164 in osteo-chondroprogenitors caused bone malformations that largely pheno-copied the effects of constitutive β-catenin activation. Juvenile or adult induction of VEGF in these cell populations also dramatically increased bone mass within

2 weeks, associated with enhanced angiogenesis. However, the bone architecture was severely disrupted by disorganized and aberrant vascularization, excessive ossification obliterating the marrow cavity, and severe cortical bone remodeling. In addition, VEGF164 induction in the bone micro-environment of adult mice caused pronounced bone marrow fibrosis and hematological anomalies. Cellular analysis revealed that VEGF over-expression altered osteoblast proliferation, differentiation and activity in vivo, and led to region-specific alterations in osteoclast activity. Mechanistically, genetic and pharmacological interventions indicated that VEGF increased bone mass via a VEGFR-2-, PI3-kinase/GSK3β- mediated pathway inducing β-catenin transcriptional activity in endothelial and osteoblastic cells. Consequently, several β-catenin target genes were upregulated in bones of VEGF over-expressing mice, contributing to the high bone density and bone marrow niche disruption. These insights into the actions of VEGF in the bone and marrow environment underscore its power as pleiotropic bone anabolic agent but also warn for caution in its therapeutic use. Moreover, the finding that VEGF can modulate β-catenin activity may have widespread physiological and clinical ramifications.

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IMMUNOEXPRESSION OF C-JUN, C-FOS, C-MYC AND MSX2 DURING ENDOCHONDRAL OSSIFICATION IN MICE

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Matrix metalloproteinases are zinc-dependent endopeptidases that degrade all components of extracellular matrix. They are able to remodelate the ECM during normal developmental processes such as embryogenesis and organogenesis, as well as in pathological processes such as tumoral invasion. The biological mineralization research looking for discovering the genes involved in the molecular mechanisms that control the endochondral ossification process. MMPs and their inhibitors (TIMPs and RECK) are responsable for bone matrix remodeling and, probably, determinate the level of its turnover. They are regulated by the same transcription factors, such as c-jun, c-fos and c-myc. MSX2 is a homeobox-contains gene important for a limb development. Our previous studies indicated a differential expression of MMPs -2 and -9, RECK, TIMPs -1 and -2 during bone formation. Thus, our aim was investigated the temporal-spatial expression these transcription factors during endochondral ossification in mice. Femurs (n=5/period) were collected from foetuses (E13-E20) and 1 day postnatal (PN1) and processed for immunohistochemistry . In early stages (E13-E15), c-fos and c-myc no were immunolabeled, but c-jun was localizated in osteoblast-like cells at the center of cartilaginous anlagen. In later stages (E18-PN1), c-myc, c-fos and c-jun were immunopositives, mainly, in osteoblasts of ossification front and in hyperthrophic chondrocytes. MSX2 was found in all periods evaluated in osteoblasts, showing intense immunostain at E15 and its expression were decreased throughout this process. Taken together, we suggested that these transcription factors maybe are important to regulate the transcription of MMPs and their inhibitors during bone development.

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CROSS-TALK BETWEEN CTGF AND TGF-BETA1 IN MESENCHYMAL STEM CELL CONDENSATION

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Condensation or the aggregation of mesenchymal stem cells (MSCs) precedes chondrocyte differentiation and is required for cartilage formation. CTGF is a matricellular protein that has been found to be expressed during MSC condensations in vivo. It has been shown that TGF-β1 regulates CTGF expression and that CTGF acts as a downstream mediator of TGF-β1 effects on extracellular matrix production. It has also been reported that CTGF has the ability to bind TGF-β1 and modulates its effects. Using C3H10T1/2 MSCs as a model for mesenchymal condensation, we have shown previously that TGF-β1 induces MSC condensation in vitro associated with increased matrix production, proliferation and migration and this induction is mediated by CTGF. In this study, we were interested to examine whether CTGF overexpression can mediate MSC condensation in the absence or presence of TGF-β1. C3H10T1/2

MSCs were infected with adenovirus over-expressing CTGF tagged with GFP achieving a 6-7 fold increase in CTGF mRNA and protein expression. Adenovirus expressing only GFP was used as control. Cells overexpressing CTGF did not show any MSC condensation. Surprisingly, TGF-β1 induced MSC condensation was inhibited in cells overexpressing CTGF. These results suggest that a fine equilibrium of CTGF expression is required for TGF-β1-induced MSC condensation. We next examined the effect of CTGF overexpression on MSC adhesion and spreading associated with vinculin localization at focal adhesion and actin cytoskeletal reorganization. Cells overexpressing CTGF spread more robustly with an increased punctuated signal of vinculin at sites of focal adhesion with the formation of lamelopodia when compared to cells infected with GFP virus. We next examined the signaling pathway associated with MAP Kinase family to evaluate differences between TGF-β1-induced MSC condensation and the inhibitory effect of CTGF overexpression on MSC condensation. Phosphorylated P38, Jnk and Erk were increased in the GFP-infected MSCs treated with TGF-β1. However, MSCs infected with GFP-CTGF and treated with TGF-β1 showed only an increase in phosphorylated Jnk and Erk but not P38. These findings indicate that p38 MAPK may mediate MSC condensation by TGF-β1. Further studies are warranted to modulate P38 expression to elucidate the interaction between CTGF and TGF-β1 in regulating MSC condensation.

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SKELETAL PHENOTYPE IN TRANSGENIC MICE OVER-EXPRESSING CTGF IN CELLS OF THE OSTEOBLAST LINEAGE

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CTGF has recently emerged as an important growth factor in osteogenesis, demonstrated by its ability to promote proliferation, matrix production and differentiation in cultures of osteoblasts. Since most of the data concerning the role of CTGF in osteogenesis has come from in vitro studies, in this study we generated transgenic mice in which CTGF is over-expressed under control of the truncated 3.6kb collagen type I (pOBCol3.6) promoter (CTGF pOBCol3.6 mice). This promoter was chosen because it is expressed early during osteoblast differentiation. The targeting vector used to generate transgenic mice also contained LacZ (to identify cells expressing the transgene) and an enhancer element to boost CTGF expression. The presence of the transgene was determined by PCR of tail DNA using transgene specific primers. Six lines were established by mating founder mice with C57/Blk6 wild type (WT) mice. Multiple tissues were used to examine specificity of transgene expression using PCR with transgene specific primers, followed by confirmation of CTGF mRNA expression levels by Northern blot analysis. Transgene expression was highest in long bone and calvaria, with lower levels of expression in other type I collagen producing tissues (lung and skin). Two of the transgenic lines with different CTGF expression levels were used for analysis of the skeletal phenotype. Mice from one line survive, however, mice from the other line die within a few days after birth. Line one showed a 3-4 fold (moderate expression) increase and line two showed a >7-8 fold (high expression) increase in CTGF protein levels in bone when compared to age matched WT mice. Histological and morphometric examination of the distal femoral metaphysis from TG mice with moderate over-expression of CTGF exhibited significant increases in trabecular bone volume associated with increased osteoid thickness and osteoblast activity/numbers compared to WT mice. Increased thickness of the periosteum with increased numbers of osteoprogenitor cells was also observed in TG compared to WT bone. Primary cultures of osteoblasts derived from these TG mice also exhibited enhanced differentiation (ALP staining and mineralization) compared to WT cultures. Surprisingly, examination of bones from transgenic mice over-expressing CTGF at very high levels demonstrated an increase in osteoclast number and size. These data suggest that the precise effects of CTGF on bone cell differentiation and function depend on the magnitude of CTGF over-expression. Moderate levels of CTGF have a direct effect on osteoblasts to promote bone formation, while high levels favor the formation of osteoclasts, perhaps indirectly through a RANK-L dependent mechanism.

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DEVELOPMENT OF THREE-DIMENSIONAL CULTURES FOR ASSESSMENT OF CELL PROLIFERATION AND OSTEOGENIC DIFFERENTIATION IN VITRO

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Bone is a complex tissue that is three dimensional in nature and capable of bearing mechanical stress. It is very difficult to simulate the characteristics of this tissue in vitro in monolayer cell culture. Therefore, reliable and stable three-dimensional (3D) systems for in vitro osteogeneic assessment are critical to study the behaviour of this tissue under different treatments in order to develop potential therapeutic strategies. The aim of this study was to develop 3D systems for evaluation of cell proliferation and osteogenic differentiation in vitro. The following 3D scafflolds were investigated: collagen scaffolds with or without channels, collagen/hydroxyapatite scaffolds, coralline hydroxyapatite and hydroxyapatite microspheres. Human bone marrow mesenchymal stem cells (hMSCs) and a murine osteoblastic cell line (2T3) were used to establish cultures on these scaffolds. Cells were seeded on these scaffolds and cultured in alph-MEM supplemented with FBS, beta-GP and asc-2P) for differentiation. Lactoferrin, an anabolic glycoprotein extracted from milk, was used to treat the cells in order to evaluate its effect on the 3D cell cultures. The cultures were assessed for cell proliferation using Alamar Blue, cell viability using Live/Dead staining, osteogenic differentiation using alkaline phosphatase and mineralisation using Alizarin Red S or Calcein. The 3D cultures were imaged with various bioimaging technologies, including laser confocal microscopy, fluorescent microscopy with Z-scan and extended focus, scanning electron microscopy and image analysing software for quantitative analysis. Collagen scaffolds with multiple channels demonstrated better penetration of cells into the scaffolds. Both coralline hydroxyapatite scaffolds and the hydroxyaptite ceramic microspheres supported cell proliferation and osteogenic differentiation, and the latter scaffolds have potential for large scale 3D dynamic culture, in which microspheres are slowly rolled in a rock-and-roll system. Lactoferrin-treated osteoblastic cells in microsphere culture, demonstrated significant increases in proliferation (3 fold) of 2T3 cells at day 3 ($p<0.05$) in non-osteogenic culture medium compared to vehicle controls.

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ANABOLIC AND ANTI-CATABOLIC SYNERGY IN BONE TISSUE ENGINEERING

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Introduction

Recombinant human bone morphogenetic protein-7 (rhBMP-7) is an anabolic bone drug used clinically for the treatment of bone defects. We have previously published that when given systemically, anti-resorptive (anti-catabolic) bone drugs such as bisphosphonates can act synergistically in a critical defect model1 . Local delivery of bisphophonates has been proposed to be deleterious for bone tissue engineering applications, due to toxic effects2. We have explored the potential synergy between rhBMP-7 and the biphosphonate Pamidronate (PAM) when both are delivered locally via a polymer pellet surgically implanted in the hind limb of a mouse.

Methods

Poly-D,L-lactic acid polymer (PDLLA) pellets containing 25µg rhBMP-7 +/- increasing doses of PAM were produced under sterile conditions. These were implanted into the hind limb muscle of 11wk old fe male C57BL6 mice to induce ectopic bone over 3 weeks. Bone formation was assessed by radiography (27kV) and quantitative computed tomography (QCT).

Results

X-ray data confirmed that local PAM could augment rhBMP-7 induced bone formation (Fig 1B-D) compared to controls without PAM (Fig 1A). However, the highest dose of PAM at 2mg/mouse produced a negative effect on BMP-induced bone formation (Fig 1E), and was confirmed by QCT scanning.

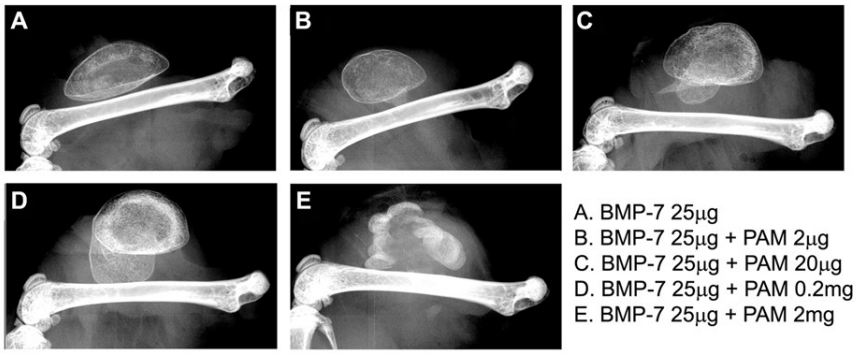

Figure 1. Radiographs of bone nodules

Discussion

The primary action of bisphosphonates (e.g. PAM) is to inhibit bone resorbing cells (osteoclasts). We speculated that PAM would antagonise the pro-osteoclastic effects of rhBMP-7 and thus maximise its pro-anabolic effect. However, at high doses, bisphosphonates can have non-specific effects that affect other cell types. Thus it was speculated that while low doses of PAM may augment rhBMP-7 induced bone, high doses may affect cells other than osteoclasts and suppress bone formation. This is what was observed in our model; PAM increased bone formation in a dose-dependent manner up until 0.2mg. Notably, the abnormal bone seen in the 2mg treatment group was comparable to the dose range used by Choi et al.2 who reported inhibited bone formation with local PAM treatment in a skull defect mode.

(1) Little et al. J Bone Miner Res. 20:2044-52 (2005)

(2) Choi et al. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 103:321-8 (2007)

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THE RELATIONSHIP BETWEEN PENTOSIDINE AND INDICES OF ARTEROSCLEROSIS IN PRE-MENOPAUSAL JAPANESE WOMEN

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Advanced glcation end products (AGE) in collagen have been reports to decrease the mechanical property of bone. Collagen cross-linking can divided into two types ; enzymatic and nonenzymatic. The enzymatic formation of cross-links may have beneficial effect on bone strength. On the other hand, nonenzymatic formation of cross-links may have effect on arterial stiffness and bone fragility .

The purpose of this study was to elucidate the relationships between pentosidine (one of the AGE) and indices of arteroscleroses (total cholesterol in serum and arterial stiffness) in pre-menopausal Japanese women.

The subjects studied were 66 pre-menopausal women at the mean age of 45.6years (37-52yrs). The height, body weight and BMI were measured as physical parameters. Previous disease, menstrual status and the age of menarche were determined using a questionnaire form. The bone mass of calcaneus was measured by using ultrasounds bone densitometer (AOS-100, ALOKA Co., Japan) . The arterial stiffness was measured by using cardio-ankle vascular index (CAVI : VaSera VS-1500N, Fukuda Denshi Co., Japan) . The CAVI has been recently reported as a new index of arterial stiffness, which is less influenced by blood pressure than pulse wave velocity (PWV). In addition, we investigated total cholesterol and calcium in serum, deoxypyridinoline in urine as a marker of bone resorption and bone alkaline phosphatase in plasma as a marker of bone integration.

The subjects were divided into two groups, a higher pentosidine group (n=27) and a lower pentosidine group (n=39). The average of total cholesterol and calcium in serum and deoxypyridinoline in the urine were higher in the higher pentosidine group than those in the lower group, while no difference was observed in bone mass of calcaneus and arterial stiffness (CAVI).

These results suggested that increased pentosidine level is a possible case of progression of arteroscleroses and bone resorption in pre-menopausal women.

VISCERAL ADIPOSITY IS INVERSELY ASSOCIATED WITH BONE MINERAL DENSITY

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Backgrounds: It has long been thought that obesity protects against osteoporosis. However, recently published epidemiologic studies challenged this thought, and showed that after control for body weight, obesity *per se* was inversely correlated with bone mineral density (BMD), and also associated with higher risk of non-spine fractures. Meanwhile, another clinical study showed that after adjusting for body mass index, metabolic syndrome was also associated with lower BMD, and higher incidence of osteoporotic non-vertebral fractures. Thus, we hypothesized that visceral obesity as well as body composition might be associated with BMD.

Methods: A total of 427 subjects (268 men and 159 women), who visited Severance hospital for medical checkup, were included in this study. The body composition was measured using the bioelectrical impedence analysis methods. The ultrasonography was performed to measure subcutaneous and visceral fat thickness. BMD was measured using dual energy x-ray absorptiometry.

Results : The mean age of participants was 55.5 ± 8.6 years for men, and 55.1 ± 9.8 for women. The mean BMI was 25.1 ± 2.6 for men, and 22.7 ± 3.1 for women. After adjusting for covariates including age, weight, regular alcohol consuming, regular exercise and postmenopausal state (in women), percent lean mass was positively associated with BMD at spine (r = 0.214, P =0.002 in men; r = 0.254, P =0.008 in women), femur neck (r = 0.203, P =0.002 in men; r = 0.166, P =0.077 in women), and total hip (r = 0.181, P =0.005 in men; r = 0.212, P =0.026 in women), whereas percent fat mass was inversely correlated with BMD at spine (r = -0.203, P =0.002 in men; r = -0.175, P =0.059 in women), femur neck (r = -0.195, P =0.002 in men; r = -0.172, P =0.061 in women), and total hip (r = -0.149, P =0.018 in men; r = -0.191, P =0.039 in women). Visceral fat thickness also showed negative relationship with BMD at spine (r = -0.159, P =0.017 in men; r = -0.275, P =0.004 in women), femur neck (r = -0.176, P =0.005 in men; r = -0.273, P =0.004 in women), and total hip (r = -0.139, P =0.028 in men; r = -0.255, P =0.008 in women), but subcutaneous fat thickness was not associated with BMD at those sites.

Conclusion: Lean mass may have a beneficial effect on BMD, whereas fat mass may affect BMD negatively. Not subcutaneous fat, but visceral fat also has a negative relationship with BMD.